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Forest tree seed production

J. N. Owens and M. D. Blake

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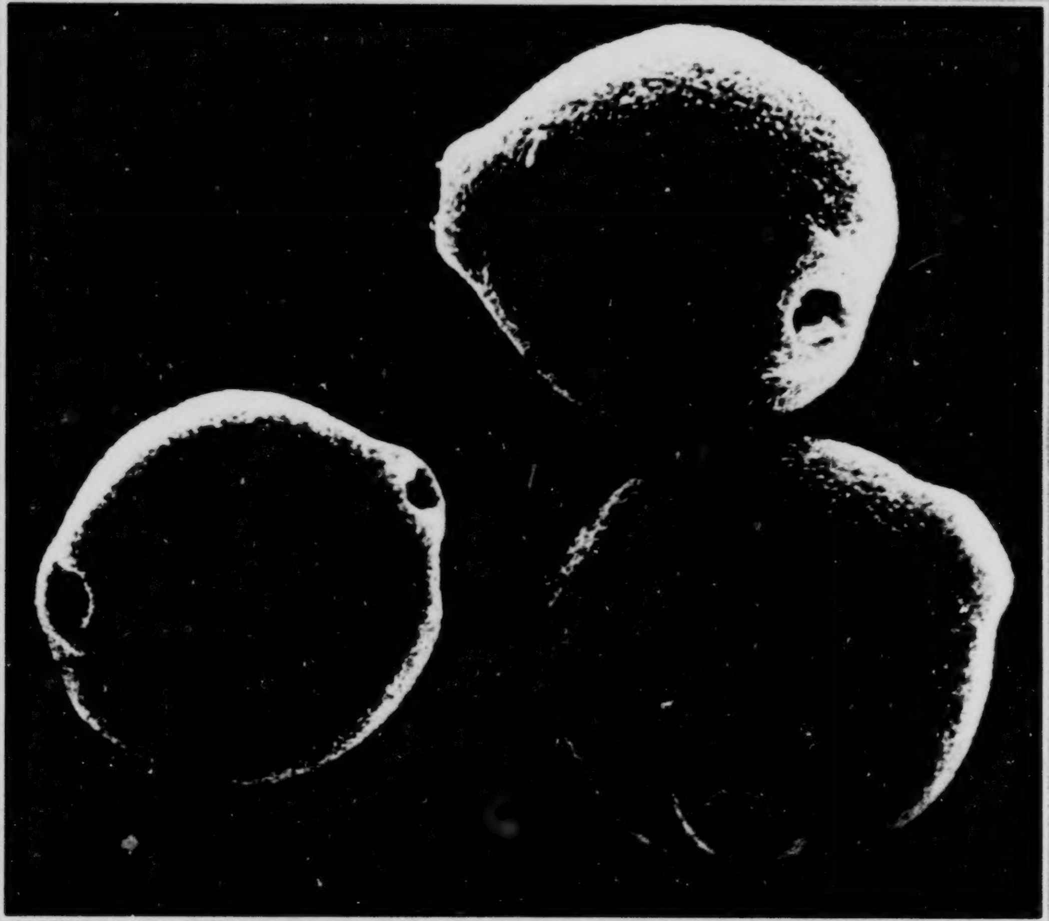
Petawawa National Forestry Institute



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COVER: Scanning electron micrograph of *Betula* pollen

FOREST TREE SEED PRODUCTION

A REVIEW OF THE LITERATURE AND RECOMMENDATIONS FOR FUTURE RESEARCH

Information Report PI-X-53

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ABSTRACT

This review describes the reproductive processes of most economically important north temperate conifer and hardwood species. It contains essential background information needed by everyone concerned with seed production. The following topics are covered within a developmental framework: (1) variation in reproductive cycles; (2) times and patterns of floral initiation; (3) environmental factors affecting floral initiation; (4) floral induction and enhancement; (5) pollen and pollination; (6) gametophyte development and fertilization; and, (7) seed development. The physiology and ecology of these processes are examined and where possible cultural, physiological, and management techniques which have been shown to affect seed production are also discussed. A summary, and recommendations for future research, concludes each chapter.

RÉSUMÉ

Cette revue décrit les processus de reproduction chez les conifères et feuillus d'importance économique dans la zone tempérée de l'hémisphère nord. Tous ceux qui s'intéressent à la production de semences y trouveront l'information essentielle de base. Les sujets, traités dans un cadre de développement, sont: (1) la variation des cycles reproductifs; (2) les temps et patrons de l'initiation florale; (3) les facteurs du milieu influençant l'initiation florale; (4) l'induction et l'accroissement floraux; (5) pollen et pollinisation; (6) le développement et la fertilisation des gamétophytes; (7) le développement des semences. L'examen de la physiologie et de l'écologie de ces processus s'accompagne, lorsque possible, d'une discussion des techniques culturales et physiologiques ainsi que des modes d'aménagement qui se sont révélés capables d'influencer la production de semences. Chaque chapitre se termine par un résumé suivi des recommandations sur la recherche à faire.

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FOREST TREE SEED PRODUCTION

A review of the literature and
recommendations for future research

INTRODUCTION

Over 5.8 billion tree seeds were used in fiscal year 1979/80 for forest regeneration across Canada (Janas and Haddon 1984). Increased emphasis on forest renewal by outplanting of seedlings and the anticipated increase in demand for forest products indicate that forest tree seed will become a major, and perhaps limiting, factor in reforestation. This will be especially true if we choose to reforest cut-over forest lands with seedlings grown from genetically improved seed.

Forest tree species usually have long juvenile periods of growth before sexual reproduction occurs, and lengthy reproductive cycles which normally include a rest period of several years duration. Therefore, there are unique problems in seed production which deserve particular attention and which may require unique approaches if seed production is to be effectively managed. The length of the juvenile period may be shortened in many species. The length of the reproductive cycle, which includes flower initiation through seed set, is a fixed period of 2 or more years which in rare instances might be shortened. More important are the many stages which occur in the long chain of events during the reproductive cycle, and the occurrence of several weak links in this chain which may result in poor seed production. We must identify those weak links which can be managed, and determine through intensive research the best methods of enhancing seed production. There is tremendous potential for increased flower production and increased seed yield. This review looks at these aspects.

We have taken a broad approach to seed production in north temperate forest trees, based on a developmental framework which puts each stage in perspective. Consequently, our review may lack depth in certain areas. Specialists in any one area will be able to identify omitted literature. It is not intended to update the specialist in his area, but to familiarize the specialist with other areas. Reviews of specific topics are cited and updated. These should be referred to for more detailed coverage of those subjects. It is considered that a broad overview may be more beneficial than yet another review of selected topics.

The following topics are examined: (1) Variation in reproductive cycles; (2) Times and patterns of floral initiation; (3) Environmental factors affecting floral initiation; (4) Floral induction and enhancement; (5) Pollen and pollination; (6) Gametophyte development and fertilization; and, (7) Seed development. Each chapter includes a description of the development of a portion of the reproductive cycle. The most relevant literature dealing with the development, physiology, and ecology of these processes are reviewed. Cultural, physiological, or management techniques which have been shown to affect seed production are examined wherever possible. A summary, and recommendation for future research, conclude each chapter. Appendices include tables which summarize results of flower induction experiments on many tree species.

This review is limited primarily to north temperate conifer and hardwood forest tree species of economic value. Tropical and subtropical species are generally not included because their reproductive cycles and factors which affect seed production may differ considerably from those of temperate forest species. Also, generally not included are fruit trees which, unlike most forest species, are either regenerated clonally or have been bred for early and reliable flowering and maximum fruit production. Research on these may not be directly applicable to forest trees, which generally have a long juvenile growth period and where rapid vegetative growth and desirable growth form are considered more important than reproductive potential.

CHAPTER 1
REPRODUCTIVE CYCLES

Introduction

The reproductive cycles of forest trees begin with the initiation of reproductive buds after a variable period of juvenile growth, which may be as little as one or as much as 40 years (Kozlowski 1971). The mature, reproductive, stage is a condition first called "ripeness to flower" (Klebs 1913), and the transition to this stage is known as "phase change" (Wareing 1964).

Grainger (1938), after studying a large number of species, distinguished three classes of temperate-zone plants based on floral initiation and development. Direct-flowering plants initiate flowers, and development through anthesis occurs without interruption. This is the most common class among herbaceous plants, and includes some woody perennial plants. Indirect-flowering plants have a period of rest (dormancy) at some stage between floral initiation and anthesis. This class includes some herbaceous plants, most woody perennials, and nearly all temperate-zone forest trees. Cumulative-flowering plants form floral primordia over a long period of time but anthesis occurs quickly. This class includes many herbaceous weed species.

Forest trees may be monoecious or dioecious. Monoecious species have male (pollen-bearing) and female (ovule-bearing) elements on separate reproductive structures borne on the same individual. Dioecious species have male and female elements on different individuals. In north temperate zones, most conifers are monoecious, while most hardwood forest species are dioecious. Some hardwood forest trees have perfect flowers with both stamens and carpels within the same flower. This hermaphroditic condition does not normally occur in conifers although bisporangiate strobili have been reported in many species.

Matthews (1963) was the first to generalize that, in temperate-zone trees, reproductive buds are initiated in the growing season preceding the spring in which cones or flowers appear and anthesis occurs. This generalization has held over the years with few exceptions (Ch. 2). Reproductive buds undergo early development before winter dormancy and overwinter at various stages (Ch. 2). During the second and, in some species, the third or fourth growing season variations occur affecting the length of the reproductive cycle (Ch. 5 and 6).

Three primary types of reproductive cycles represent the variation found in most temperate-zone forest trees. Less is known about the phenology of hardwood reproductive cycles than that of conifers.

The 2-year cycle

The most common reproductive cycle in conifers and hardwoods is represented in Fig. 1.1, depicting Picea glauca (Owens and Molder 1984c). Pollination occurs in the spring or early summer of the second year. The time between pollination and fertilization is brief, usually only a few weeks. Following fertilization, embryo and seed development are rapid and continuous. Seeds are mature and may be released as early as late summer the year of pollination. Retention of seed beyond that time is often determined by climatic or biotic requirements unique to a species and by its method of seed dispersal. Although this is the shortest reproductive cycle generally found in forest trees, it takes a long time compared to many direct-flowering herbaceous plants in which the entire cycle may be completed within a few weeks (Leopold and Kriedemann 1975).

Detailed descriptions of the complete reproductive cycles of conifers with this pattern are few and include Larix (Owens and Molder 1979 b,c), Picea (Owens and Molder 1984c), Pseudotsuga (Allen and Owens 1972, Owens 1973), Thuja (Owens and Molder 1984a) and Tsuga (Owens and Molder 1984d). There are descriptions of portions of the reproductive cycles of many species and several will be included in subsequent chapters.

The 3-year-cycle - I

A second reproductive cycle is found in most species of Pinus, several other conifers, and a few hardwoods. Unfortunately, this cycle is used in general textbooks as the "typical" conifer reproductive cycle (Fig. 1.2). Pollination occurs in the spring or early summer of the second year; pollen tube and ovule development is initiated but then stops, usually in mid-summer.

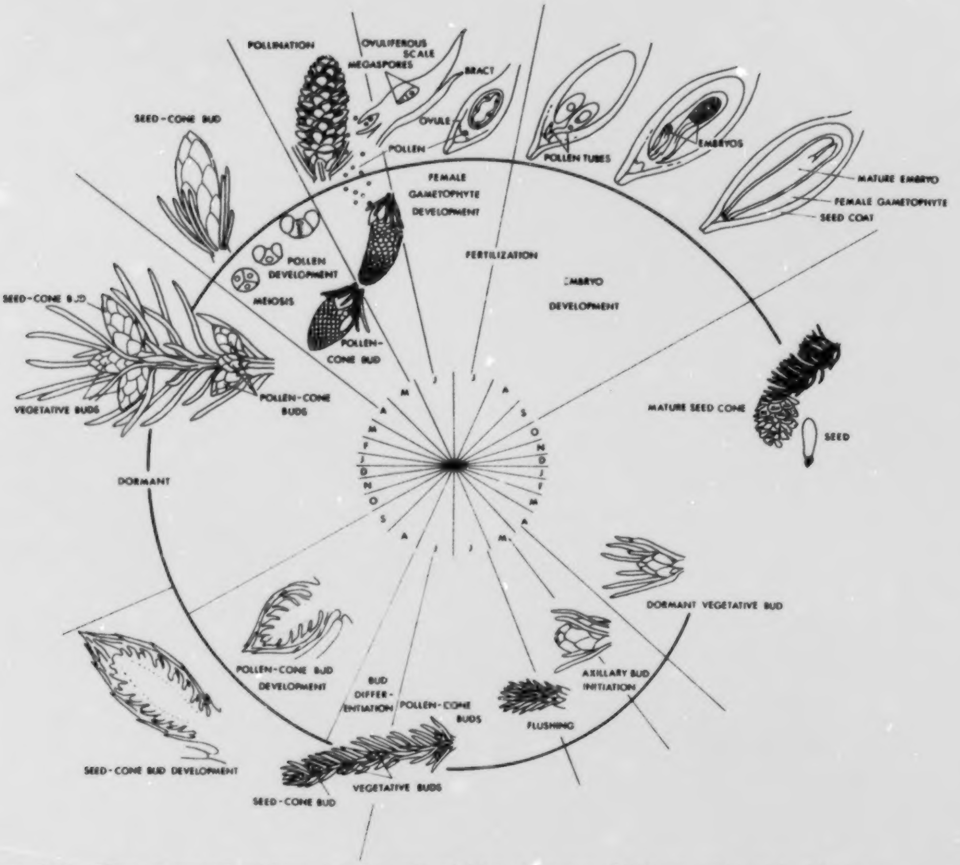

Figure 1.1 The reproductive cycle of white spruce (Picea glauca) (from Owens and Molder 1984c).

Development resumes the following spring. Fertilization occurs and embryos and seeds are mature by the fall. Seeds are usually shed in the year they mature. Serotinous seed cones of some species may remain closed for many years. They usually open in response to extreme heat from fires and release many years of accumulated seeds at one time. This minimum 3-year cycle, commonly about 27 months from reproductive-bud initiation to seed maturity, also occurs in southern hemisphere conifers such as the Araucariaceae, including Araucaria (Favre-Ducharte 1962) and Agathis (Eames 1913); the Podocarpaceae, including several species of Podocarpus (Looby and Doyle 1944a, b), Saxegothaea (Looby and Doyle 1939), Dacrydium (Quinn 1966a, b), and Widdringtonia (Schnarf 1933, Moseley 1943). A few less familiar northern hemisphere genera also have a cycle similar to Pinus, cycle similar to Pinus, including Sciadopitys (Lawson 1910, Buchholz 1931, Tahara 1937, 1940, Gianordoli 1964), Sequoia (Buchholz 1939a, b), and Cephalotaxus (Singh 1961). Descriptions of many of these genera are brief or cover only a few aspects of the reproductive cycle. Complete descriptions of this type of reproductive cycle are limited to Pinus (Lill 1974, Owens and Molder 1984b). There are no detailed descriptions of hardwoods having this type of reproductive cycle (see Fowells 1965).

The 3-year-cycle - II

A third reproductive cycle is found in a few conifers in the Cupressaceae family. Pollination occurs in the spring or early summer of the second year, and fertilization occurs within a few weeks. Embryo and

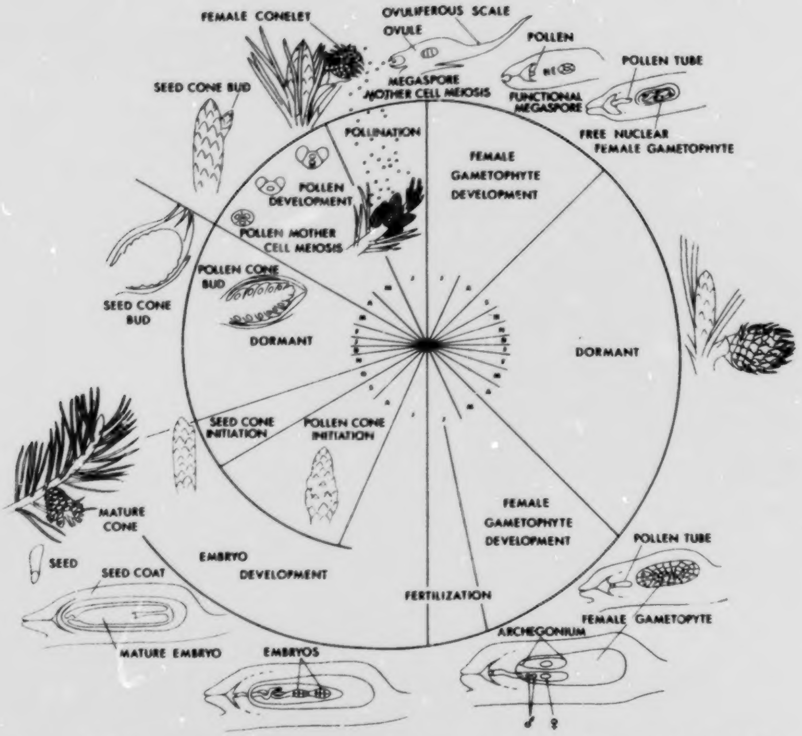

Figure 1.2 The reproductive cycle of lodgepole pine (Pinus contorta) (from Owens and Molder 1984b).

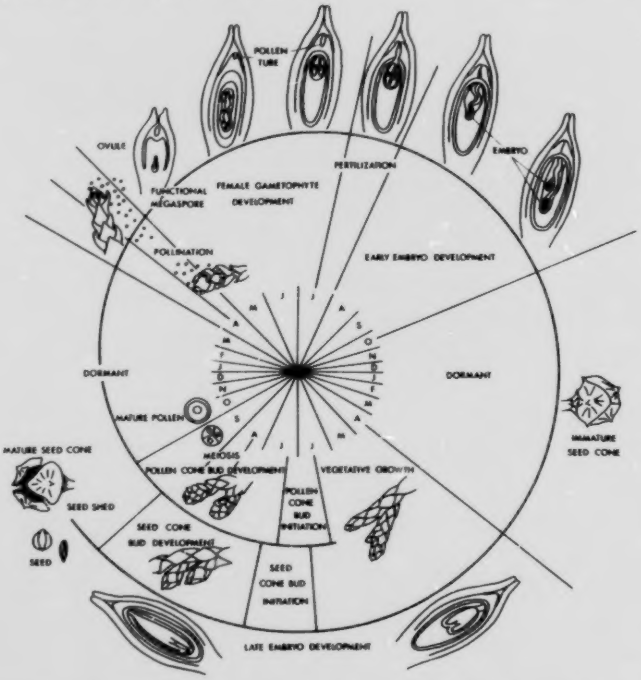

Figure 1.3 The reproductive cycle of yellow cedar (Chamaecyparis nootkatensis) (from Owens and Molder 1984a).

seed development begins but become arrested in late summer or fall. The seeds and cones overwinter in a dormant condition, then resume development in the spring of the third year (Fig. 1.3). This reproductive cycle has been completely described for Chamaecyparis nootkatensis (Owens and Molder 1984a) and partially described for several species of Juniperus (Johansen 1950). Extended indirect-flowering species such as these and those mentioned for the previous reproductive cycle may have arisen as an adaptation to short growing seasons. Embryo and/or seed development and cone development occur over two (or perhaps more) growing seasons.

Variations of the basic cycles

General silvics (Fowells 1965) and seed manuals (Schopmeyer 1974) refer to the time between pollination and seed release. However, where this time extends over more than one year the reason is not given. This is particularly true for hardwoods, some of which have 2-year reproductive cycles. In these cases it is usually uncertain if the extended cycle results from the second or third type of life cycle.

A 2-year cycle which is intermediate between the normal 2-year cycle and the 3-year cycle of Pinus occurs in the Western Himalayan Cedrus deodara (Roy Chowdhury 1961). Flowers are initiated in the summer and pollination occurs in the fall of the same year but fertilization does not occur until after winter dormancy. Seeds mature late in the second year.

A combination of the two 3-year reproductive cycles occurs in Juniperus communis (Ottley 1909, Kotter 1931) and three species of Pinus (pinea, leiphylla, and torreyiana) (Dallimore and Jackson 1966, Francini 1958). In J. communis, flower initiation occurs before winter dormancy and pollination occurs the following spring. Pollen tube growth and ovule development become arrested and overwinter, with fertilization occurring in the third year. The immature embryos overwinter, then complete development during the fourth growth season. In the three species of pine, pollination occurs in the spring but pollen tube and ovule development remain arrested for two years. Fertilization, and embryo and seed maturation occur in the fourth year.

Summary, and recommendations for future research

In the long reproductive cycles which occur in conifers and some hardwoods, there is tremendous scope for variation in phenology, even though the sequence will remain unchanged. We must not assume that a species fits the textbook example, especially if we are attempting to control seed production. Also, as the length of the reproductive cycle increases so does the possibility of something going wrong. Therefore, it is not surprising that many species have rather low seed yields. To determine the causes of poor seed yield we must determine what went wrong at what stage of development.

Complete descriptions of life cycles of all forest trees or even brief mention of those for which the phenology is known is beyond the scope of this review. Researchers undertaking seed production studies should refer to standard references such as "Silvics of Forest Trees of the United States" (Fowells 1965, now being revised) or "Seeds of Woody Plants in the United States" (Schopmeyer 1974) for general information on time from pollination to seed maturity. Specific aspects of reproduction will be dealt with in subsequent chapters.

The limited descriptions of the times of pollination and seed maturity given in silvics and seed manuals may be adequate for estimating potential seed crops and timing of seed collections in the field. However, they are inadequate for seed orchard management and genetic tree improvement programs. The lengthy reproductive cycles of many forest species provides a long period for adversities to affect seed yield. To understand factors affecting seed yield we must first be aware of the many individual steps which lead to mature viable seed. The complexity of this developmental process is indicated by the three basic reproductive cycles given above. It may be expedient but unwise to make assumptions about a reproductive cycle.

It is recommended that, before genetic tree improvement and seed production programs for a species get too far underway, the complete reproductive cycle of the species be studied under natural conditions and also under seed orchard conditions if the latter is removed from the natural distribution of the species. Failure to do this may be more costly in wasted time and resources over many years than the cost of undertaking a fundamental study of the reproductive cycle. These studies should include the time and method of reproductive bud initiation (Ch. 2), pollen development and the pollination mechanism (Ch. 5), fertilization (Ch. 6), and embryo, seed, and cone or fruit development up to the time of seed maturity and release (Ch. 7). These studies are not difficult but often fall outside the experience of many forestry laboratories. This is an opportunity to utilize the expertise of botanists for the benefit of forestry.

CHAPTER 2

FLORAL INITIATION

Introduction

The term "floral initiation" is used to describe the transition of an indeterminant vegetative apical meristem (apex) or an undetermined axillary apex into a determinant reproductive apex that may develop into angiosperm flowers or conifer strobili (cones). The term describes a process, rather than a specific structure.

Floral initiation is the first step in any reproductive cycle. There have been several reviews on flowering, emphasizing floral initiation (Hillman 1962, Searle 1956, Chailakhyan 1968, Evans 1969a, b, 1971, Bernier 1971, Zeevart 1976, Halevy 1985). The long and futile search for a flowering hormone "florigen" has centered around this stage of development (Evans 1971). Early arguments for a single floral initiation substance were derived from the work of Chalakhyan (1936a, b, c, 1937) and others (see Hillman 1964) on herbaceous angiosperms that flowered in response to photoperiod. Arguments against a single hormone that induces flowering in all plants center around the tremendous variation between species and the conditions under which they flower (Evans 1969b). Another argument against such a hormone is that long-lived plants, such as woody perennials, would not survive if flowering were under the control of a single stimulus (Romberger and Gregory 1974). Evans (1969b) and Jackson and Sweet (1972) proposed as an alternative that flowering in temperate woody perennials resulted from a series of developmental stages, each sequentially determined by the hormone balance at the initiation site (apex) at the appropriate time. Even this may be too simplistic a view of floral initiation in forest trees. This is not to suggest that the problem is too complex to be solved. Indeed, there are many common factors between woody perennials and herbaceous plants during floral initiation. Also, the anatomical and biochemical changes occurring during the transition of a shoot apex to a floral apex are essentially the same for angiosperms (Bernier 1971) and conifers (Owens 1980).

The time and site of initiation in conifers

Reproductive buds may be borne terminally or laterally (in axils of leaves) on the branch and, in most, except such families as the Cupressaceae and some Taxodiaceae, they are enclosed by bud scales. The buds are simple because they normally contain a single strobilus and no leaves.

In the Pinaceae, seed cones are produced first, followed by pollen cones, usually several years later (Wareing 1958, Fraser 1958). There is renewed interest in shoot development and cone position as part of quantitative studies of crown form and tree architecture (Powell 1977a, 1979, Powell et al. 1984). In general, seed cones occur on vigorous lower order shoots, whereas pollen cones occur on less vigorous higher order shoots (Wareing 1958, Debazac 1965, Baradat 1967, Varnell 1976, Powell 1972, 1977a). Similar observations have been made for the Cupressaceae (Courtot and Baillaud 1955). There are several general reviews of times and methods of flower initiation (Owens 1973, 1980, Puritch 1972, Owens and Molder 1977g, 1979d, Eis and Craigdallie 1981). Other reports contain more detailed observations and are included below with the time and method of reproductive bud initiation for separate genera. A summary is given in Fig. 2.1. For many conifers either no information is available or there is only general information (Fowells 1965).

Abies (true firs)

Several species have been studied, including A. amabilis (Ritchie 1966, Owens and Molder 1977a), A. balsamea (Powell 1974, 1977a, b), A. grandis (Owens 1984b), A. lasiocarpa (Owens and Singh 1982), A. procera (Ritchie 1966) and A. veitcheii (Seido and Osada 1979). A summary is given by Owens and Molder (1985). Potential seed cone buds are initiated in the axils of leaves on the upper surface of elongating primary or secondary shoots in the upper few whorls of the crown. Seed cone buds may occur on vigorous nodal shoots or less vigorous internodal shoots. Potential pollen cone buds are initiated in the axils of leaves on the lower surface of elongating shoots in mid- and lower regions of the crown. There is usually little overlap between seed cone and pollen cone bearing regions and seldom do the two occur on a

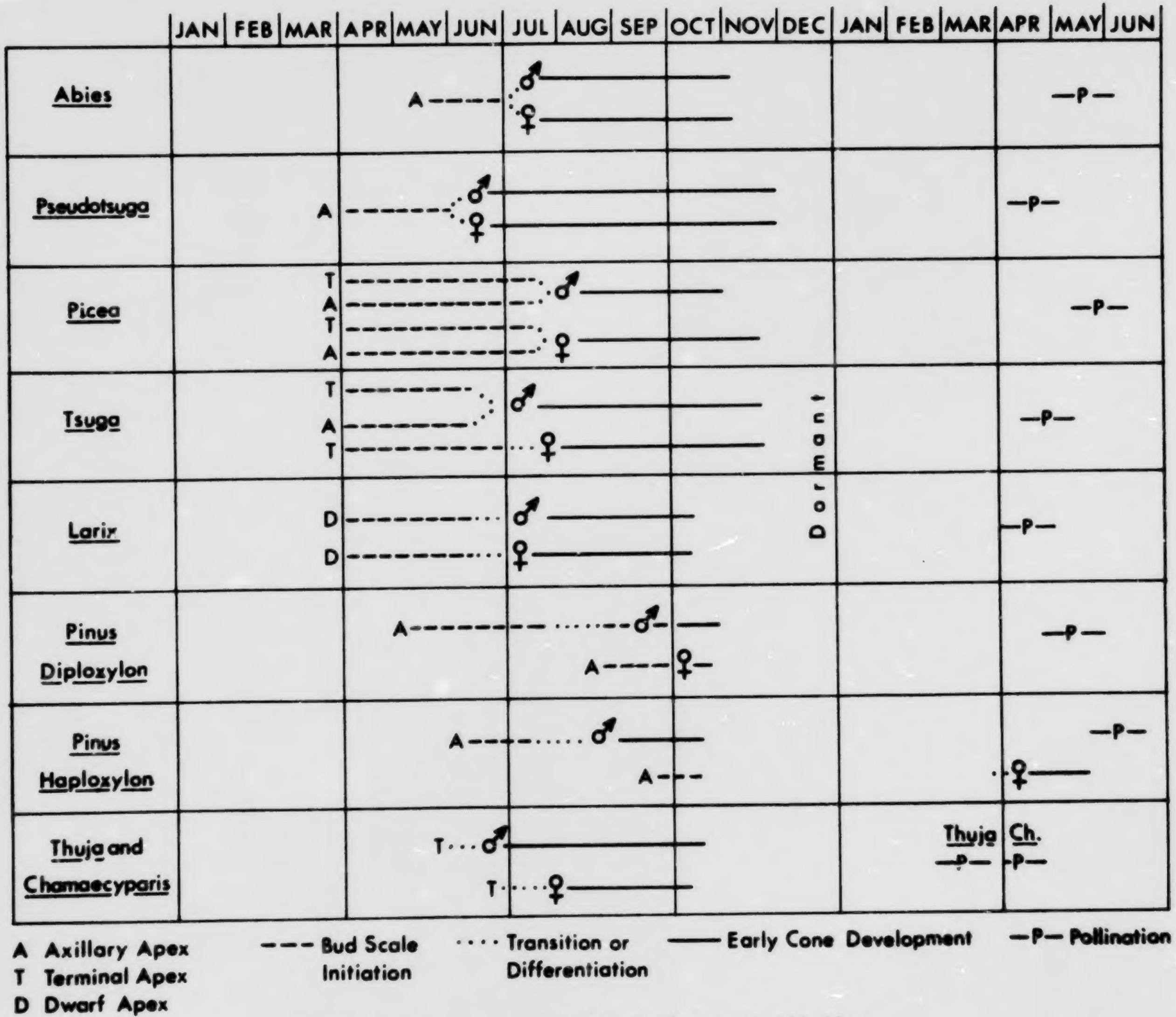

Figure 2.1 Times and methods of cone initiation.

single branch. All axillary buds are initiated during early shoot elongation, at about the time of vegetative bud flush. Axillary buds do not become determined as pollen cone, seed cone, or vegetative buds until the end of bud scale initiation. Microsporophylls, bracts, ovuliferous scales, and leaves begin to be initiated about mid-July. Pollen cone buds complete development in about two months, whereas seed cone and vegetative buds continue development into autumn. All microsporophylls, bracts, fertile ovuliferous scales, and leaves are initiated before winter dormancy. In addition to the above, three alternative pathways of bud development, axillary buds may abort during early development or become latent before becoming determined (Fig. 2.2). Aborted buds often degenerate before forming many bud scales, whereas latent buds initiate many bud scales and retain a living apical meristem capable of future growth.

Pseudotsuga (Douglas-fir)

P. menziesii has been studied extensively (Owens and Smith 1964, Owens 1969, Allen and Owens 1972). It is similar to Abies in most respects, except that the position of reproductive buds is less rigorous. There is considerable overlap of seed cone and pollen cone bearing regions within the crown and both types of reproductive buds often occur on the same shoot. In the latter case, seed cone buds are more distal. Both bud types occur primarily on the lateral and lower surfaces of shoots. All axillary buds are initiated at the onset of vegetative bud growth and are developing several bud scales before vegetative bud flush. The earliest stages

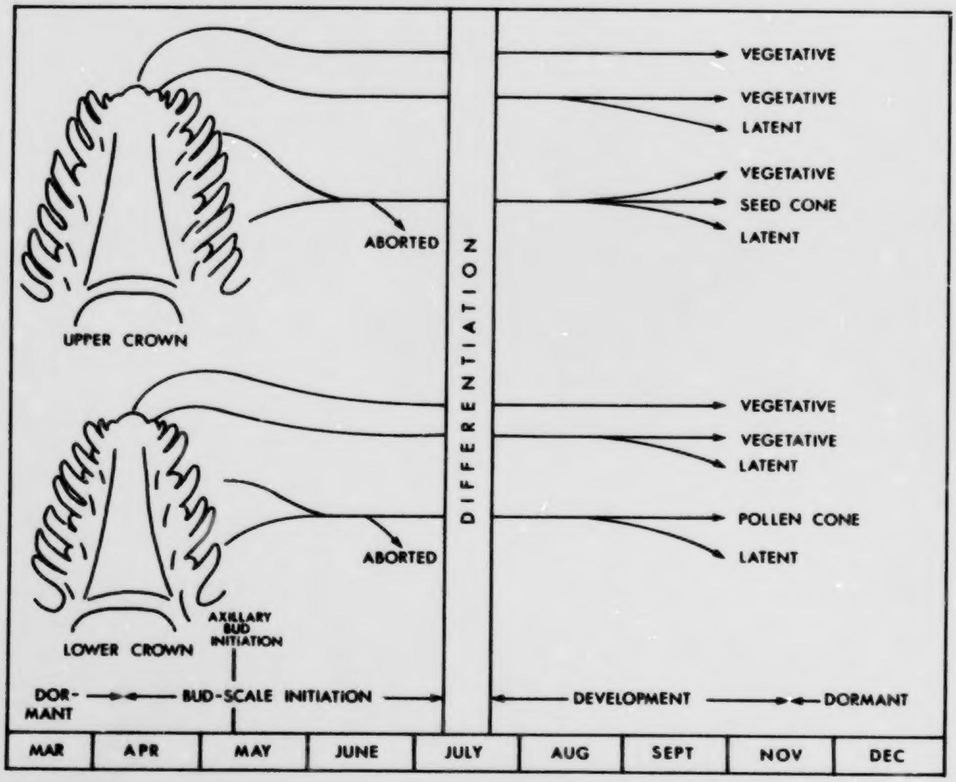

Figure 2.2 Potential pathways of terminal and axillary bud development in Abies. Lower line shows vegetative bud development (from Owens and Molder 1985).

of axillary bud determination can be recognized by using histochemical tests in early June. Axillary buds become anatomically distinct in early July (Owens 1969). Biochemical changes begin in reproductive apices during the latter stages of bud scale initiation, several weeks before anatomical changes occur. All buds become anatomically determined when lateral shoot elongation is nearly complete (Owens et al. 1985). Microsporophylls, bracts, and leaves begin to be initiated in early July. Pollen cone buds complete development by late summer. All types of buds become dormant in the fall (Fig. 2.1). All microsporophylls, microsporangia, bracts, fertile ovuliferous scales, and leaves are initiated before buds become dormant. As in *Abies*, terminal buds rarely become reproductive and axillary buds may abort or become latent. In *Pseudotsuga* the number of reproductive buds which develop is determined not by the number of axillary buds initiated (although this does vary) but primarily by the proportion of these buds which differentiate into reproductive buds (Fig. 2.3) (Owens 1969).

Picea (spruces)

The time and method of reproductive bud initiation has been determined for *P. glauca* (Fraser 1962, Eis 1967, Owens and Molder 1977e), *P. engelmannii* (Harrison and Owens 1983), *P. mariana* (Fraser 1966, G. Caron, pers. comm.) and *P. sitchensis* (Owens and Molder 1976) and is summarized by Owens and Molder (1984c). Spruce reproductive buds may develop from terminal apices which have been vegetative for one or more years or from newly-initiated axillary apices on elongating shoots (Fig. 2.4). In *P. engelmannii*, reproductive buds, when abundant, occur mostly in the axillary but also in the terminal position. When reproductive buds are few (less than 35 per cent of total buds) they are about equally distributed in terminal and axillary positions (Harrison and Owens 1983). This also appears to be true for *P. glauca* (Owens and Molder 1977e) and *P. mariana* (G. Caron, pers. comm.).

The time of bud determination is remarkably similar for most species of *Picea* which have been studied. Buds become anatomically determined at the end of bud scale initiation, which occurs near the termination of lateral shoot elongation (Owens et al. 1977, Owens and Molder 1977e, Harrison and Owens 1983, Dunberg 1979). Dunberg (1979) stressed that biochemical differentiation must occur before shoot elongation stops and anatomical differentiation begins. Pollen cone, seed cone, and vegetative buds in terminal and axillary positions become determined at essentially the same time in all parts of the

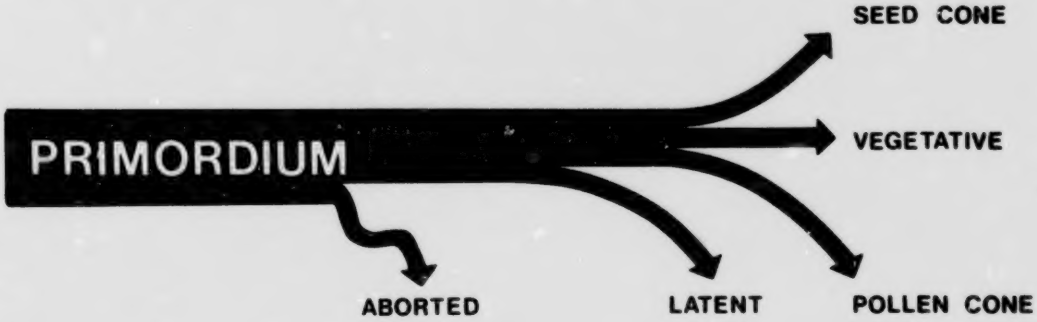

Figure 2.3 Alternative pathways of axillary bud development in Douglas-fir (from Allen and Owens 1972).

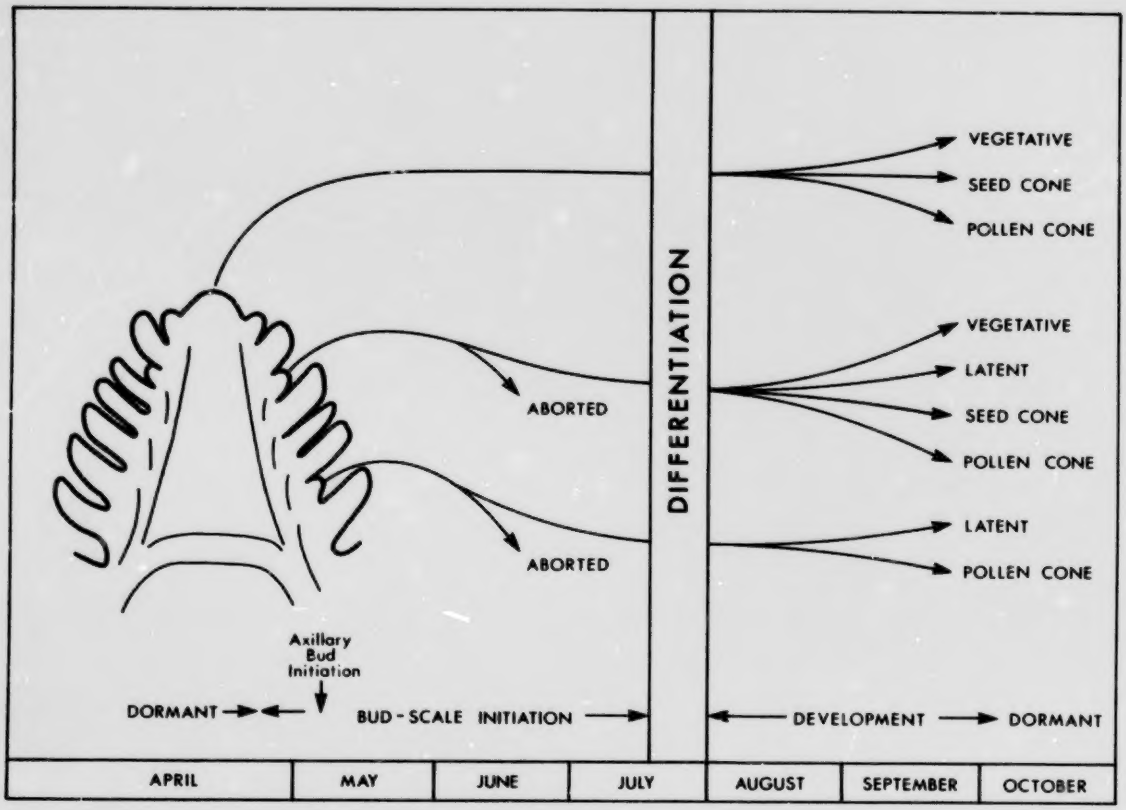

Figure 2.4 Potential pathways of terminal and axillary bud development in Picea. Lower line shows vegetative bud development (from Owens and Molder 1984c).

crown of a tree. In P. sitchensis (Owens and Molder 1976) and P. glauca growing at low elevations (Fraser 1958, Owens and Molder 1976, 1977e), bud determination begins about mid-July. In P. engelmannii growing at higher elevations determination begins in mid- to late July (Harrison and Owens 1983). Since the time of bud determination may vary with elevation and latitude, provenance differences may be significant. As in the above genera, pollen cone bud development is completed first, usually by late September or early October. Seed cone and vegetative bud development may continue until November or December in coastal P. sitchensis (Owens and Molder 1976) and mid-October in interior species (Owens and Molder 1977e, Harrison and Owens 1983) (Fig. 2.1). All microsporophylls and microsporangia, bracts and functional ovuliferous scales, and leaves are initiated before buds become dormant (Owens and Molder 1984c). Axillary buds have the same potential pathways as in Pseudotsuga (Fig. 2.3) and the abundance of reproductive buds is determined more by the pathways along which buds develop than by the number of axillary buds initiated. Terminal reproductive buds halt the future growth of a shoot, and abundant terminal cones can reduce the cone-bud production and crown expansion of a tree for several years.

Tsuga (hemlocks)

Only T. heterophylla (Owens and Molder 1974a) and T. mertensiana (Owens 1984a) have been studied and these studies are summarized by Owens and Molder (1984d). There is considerable overlap of seed and pollen cone buds in the crown and on branches. Seed cone buds are always terminal (exceptions occur following cone induction treatments) and develop from apices which have been vegetative for one or more years. They form on vigorous lateral shoots in distal portions of branches. Pollen cone buds usually develop from newly initiated axillary buds on short lateral shoots in proximal portions of branches. They commonly form a cluster of buds at the base of the shoot but the terminal apex may also develop into a pollen cone bud. Cone position is essentially the same in both species (Owens and Molder 1984d). In coastal, low elevation, T. heterophylla, pollen cone buds become determined in late June and seed cone buds in mid-July (Fig. 2.5). In coastal, high elevation, T. mertensiana, both pollen and seed cone buds become determined in late July (Fig. 2.1). Terminal, potential seed cone apices have limited pathways of development. They may remain vegetative or differentiate into seed cone buds after bud scales are initiated. Axillary, potential pollen cone buds may abort, become latent or differentiate into pollen cone buds. In T. heterophylla, reproductive buds continue development until late fall, whereas in T. mertensiana development stops by mid- to late October. All microsporophylls and microsporangia, bracts, and functional ovuliferous scales are initiated before dormancy. Prolific terminal seed cone development is common and may limit subsequent vegetative growth of branches.

Larix (larches)

In Larix, the potential pathways of axillary bud development are more limited than in previously described genera. L. occidentalis and L. laricina have been studied in detail and the position of reproductive buds is generally considered to be similar in other species of Larix (Dallimore and Jackson 1966). Both pollen and seed cone buds normally differentiate from previously vegetative terminal buds on dwarf (short) shoots that are at least one-year-old (Fig. 2.6). Reproductive buds may develop from terminal long shoot buds on suppressed branches of L. occidentalis (pers. obs.). Newly initiated buds normally develop only into dwarf shoot or long shoot buds. However, in several young trees of L. laricina, seed cone buds and, to a lesser extent pollen cone buds, differentiated from newly initiated axillary buds (Powell et al. 1984). This position has not been reported in other species of Larix. In L. occidentalis pollen cone buds are commonly proximal on nonvigorous, often pendant, long shoots. Seed cone buds are commonly distal on vigorous, but only slightly pendant to upswept long shoots. Considerable overlap occurs in distribution of reproductive buds in the crown and along branches (Owens and Molder 1979b). Pollen and seed cone buds in L. occidentalis become determined in mid-June. Microsporophyll initiation is complete in about 6 weeks and is followed by microsporangial development. Pollen cone buds become dormant in early November. Bracts and ovuliferous scales develop until early November when seed cone buds become dormant (Fig. 2.1). Vegetative buds become dormant in mid-October (Owens and Molder 1979b). Similar detailed studies have not been made for other Larix species. Related genera, Cedrus and Pseudolarix, within the Laricoideae of the Pinaceae have similar distributions of cones but details of development are not known.

Pinus (pines)

Pines were the first conifers to be studied with regard to reproductive bud initiation (Doak 1935, Sacher 1954, Duff and Nolan 1958, Gifford and Mirov 1960). Unlike the genera discussed previously, pines produce axillary bud primordia within a complex long shoot bud (LSB) rather than on an elongating shoot. Consequently, an understanding of LSB development is essential.

The LSB consists of a series of scale leaves (cataphylls) initiated throughout the growing season. Most cataphylls have an axillary apex which initiates a series of bud scales, then differentiates into a dwarf shoot, pollen or seed cone, or lateral LSB. Therefore, axillary buds are also initiated and differentiated throughout the growing season. The time of differentiation is determined by position in the LSB (Doak 1935, Sacher 1954, Duff and Nolan 1958, Owston 1969, Van den Berg and Lanner 1971, Sucoff 1971, Curtis and Popham 1972, Lanner and Van den Berg 1975, Owens and Molder 1975a, 1977b, c). LSB's may be monocyclic, consisting of one complete sequence or polycylic, consisting of two or more sequences.

Axillary buds that are initiated at the base of the LSB in the spring or early

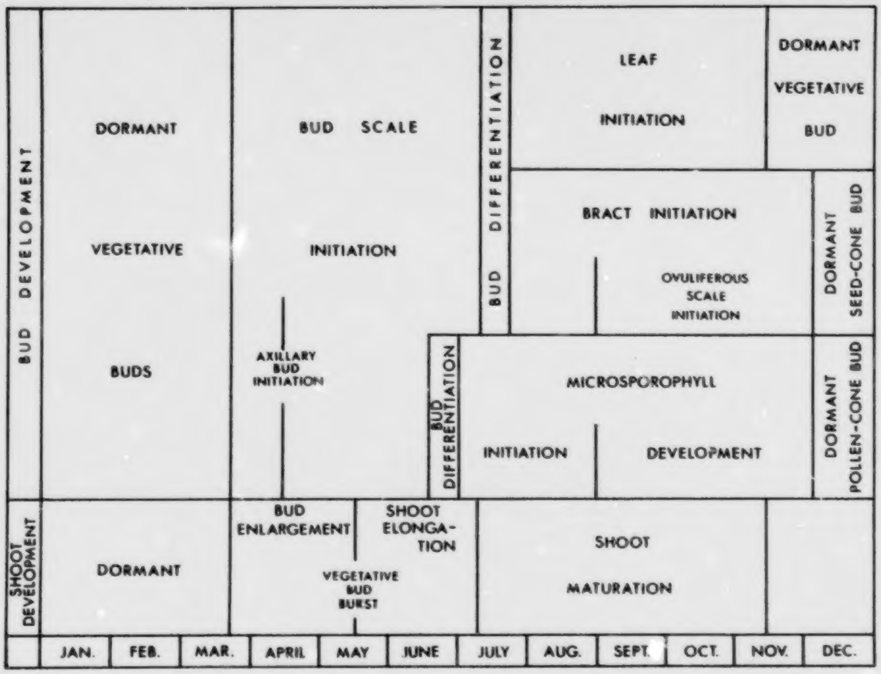

Figure 2.5 Phenology of vegetative, seed-cone, and pollen-cone bud development in *T. heterophylla* (from Owens and Molder 1974a).

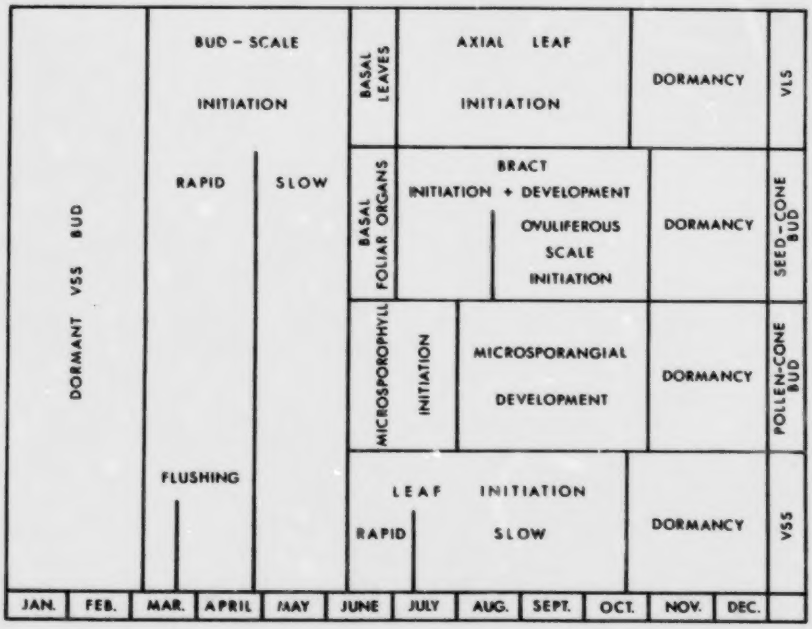

Figure 2.6 Phenology of vegetative short shoot (VSS), vegetative long shoot (VLS), pollen-cone, and seed-cone bud development in *L. occidentalis* (from Owens and Molder 1979b).

summer differentiate into pollen-cone or dwarf-shoot buds during the summer. A more distal group of axillary buds differentiate into dwarf shoots. The most distal axillary buds are initiated in late summer and after a period of bud scale initiation, differentiate into either lateral LSB or seed cone buds (Fig. 2.7). In hard pines (diploxylon), lateral LSB and seed cone buds differentiate in late summer or early fall in north temperate regions (Ferguson 1904, Shaw 1914, Sacher 1954, Duff and Nolan 1958, Gifford and Mirov 1960, Van den Berg and Lanner 1971, Curtis and Popham 1972, Owens and Molder 1975a). In soft pines (haploxylon), distal axillary primordia do not become determined before the LSB becomes dormant but differentiate immediately following winter dormancy (Fig. 2.7) (Sacher 1954, Owston 1969, Owens and Molder 1977b, c).

In pines having polycyclic LSBs, more than one series of seed cone buds may occur in a LSB but the first series usually bears most of the buds (Lanner and Van den Berg 1975, Owens and Molder 1975a, Sweet 1979, Greenwood 1980). Polycyclic LSBs occur in many hard pines and are most common in those from southern latitudes. They have not been reported in soft pines.

Because potential reproductive buds become determined over a considerable portion of the growing season, their distribution on the branch and in the crown is extremely variable depending upon the species, site, crown form, age, climate, etc. Generally, seed cone buds develop on vigorous shoots (Varnell 1976) in upper regions of the crown. They often overlap with pollen cone buds on less vigorous shoots. In some hard pines (Owens and

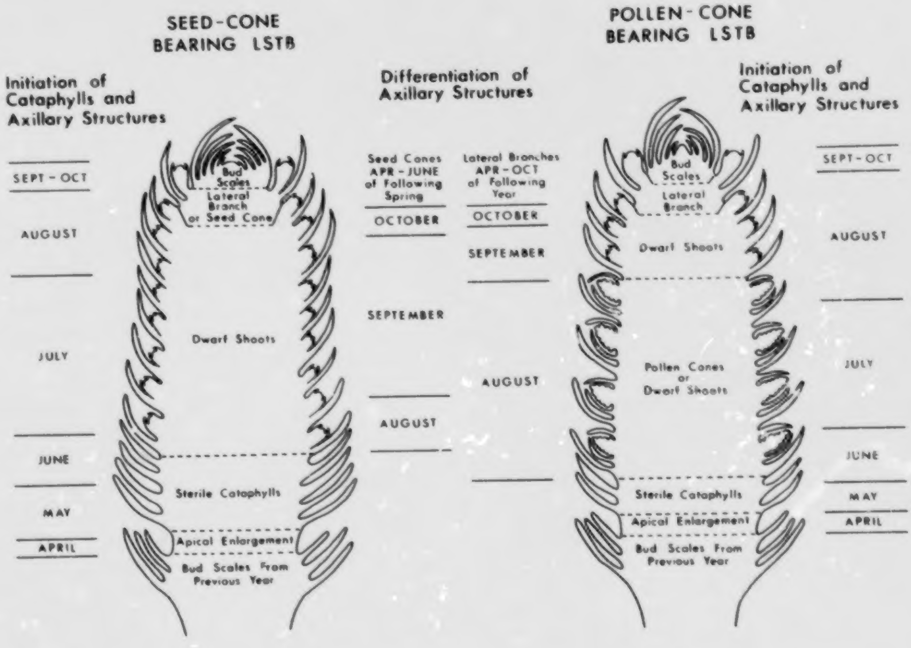
SEED-CONE BEARING LSTB
POLLEN-CONE BEARING LSTB
Initiation of Cataphylls and Axillary Structures
Differentiation of Axillary Structures
Initiation of Cataphylls and Axillary Structures
SEPT - OCT
AUGUST
JULY
JUNE
MAY
APRIL
Bud Scales
Lateral Branch or Seed Cone
Dwarf Shoots
Sterile Cataphylls
Apical Enlargement
Bud Scales From Previous Year
Seed Cones APR - JUNE of Following Spring
OCTOBER
SEPTEMBER
AUGUST
Lateral Branches APR - OCT of Following Year
OCTOBER
SEPTEMBER
AUGUST
Bud Scales
Lateral Branch
Dwarf Shoots
Pollen Cones or Dwarf Shoots
Sterile Cataphylls
Apical Enlargement
Bud Scales From Previous Year
SEPT - OCT
AUGUST
JULY
JUNE
MAY
APRIL

Figure 2.7 The development of the monocyclic LSB of P. monticola (haploxylon). The columns on the far left and far right indicate the time of initiation of cataphylls and axillary structures. The center columns indicate the approximate times of differentiation of these axillary structures (from Owens and Molder 1977b).

Molder 1975a) both types of reproductive buds occur on the same LSB and the proportion of each may vary within the crown. Variable time of determination means cone buds vary in stage of development at dormancy more than many other genera. Pollen cone buds usually form all microsporophylls and microsporangia before winter dormancy (Owens and Molder 1975a, 1977b, c). Seed cone buds may bear only a few bract primordia, or nearly all bracts and ovuliferous scales, depending upon the time between bud determination and winter dormancy.

The number of cone buds formed in *Pinus* depends on the vigor of the LSB, the number of cataphylls bearing axillary buds, as well as the pathway along which axillary buds differentiate.

Cupressaceae (cypress family)

The time and method of bud initiation has been determined only for *Cupressus arizonica* (Owens and Pharis 1967), *Thuja plicata* (Owens and Pharis 1971) and *Chamaecyparis nootkatensis* (Owens and Molder 1974b, 1984a). In the Cupressaceae, reproductive buds form by transition from vegetative apices. Buds are not enclosed by bud scales. Seed cones are terminal on short lateral shoots located on distal portions of vigorous shoots. Pollen cones are terminal on proximal, less vigorous lateral shoots. There is considerable overlap in cone distribution on branches and in the crown. Shoot elongation and leaf and axillary bud initiation occur over most of the growing season. Transition from vegetative to pollen cone apices begins in early to late June in *T. plicata*, and in late June in *C. nootkatensis*. Transition to seed cone apices follows in four weeks and one week, respectively (Owens and Pharis 1971, Owens and Molder 1974b, 1984a) (Fig. 2.1). Only pollen cone initiation as a result of gibberellin A_3 treatments, rather than under natural conditions, was studied in *Cupressus arizonica* (Owens and Pharis 1967). In the Cupressaceae which have been studied, all microsporophylls and microsporangia, bracts, scales, and ovules are initiated before cone buds become dormant in the fall. The terminal position of reproductive buds ends vegetative growth of that shoot, except pollen cone bearing shoots of *Chamaecyparis* and *Cupressus*. In these, an axillary apex is initiated at the base of the cone. After anthesis the pollen cone is shed and the vegetative shoot elongates and assumes a terminal position. This shoot could form another pollen cone in a subsequent year (Owens and Pharis 1971, Owens and Molder 1974b, 1984a). Cone initiation in the Cupressaceae is comparable to floral initiation in many angiosperms.

Other conifers

Other conifers have not been studied but it is safe to assume that in most temperate conifers, reproductive buds become determined in the spring or summer of the year before anthesis (Matthews 1963). Exceptions may occur in tropical species which have very short dormant or resting periods and slight seasonal changes. The time and method of cone initiation of a species is an important consideration in the timing of cone induction treatments (Ch. 4) and in understanding the cyclic pattern of cone production under natural conditions (Ch. 3).

The time and site of floral initiation in hardwoods

Floral initiation in hardwood trees has been studied much less and is generally more complex than in conifers. The greater complexity of hardwoods is a natural result of more diverse genera and more variation in floral bud structure. Jackson and Sweet (1972) classified six of the more common types of floral buds based on position (terminal or axillary), possession of flower parts only (simple, as in conifers), or possession of leaves and flowers (mixed buds). Mixed buds, upon expansion, produce leafy shoots terminating in a flower, or have axillary flowers and a terminal shoot that remains vegetative. In addition to being monoecious or dioecious, hardwoods may have perfect (hermaphroditic) flowers, imperfect (unisexual) flowers, or be polygamous, having both unisexual and bisexual flowers on the same plant. For most hardwood forest genera, floral development has seldom been described for more than one species. Several species within a genus may be similar morphologically but not phenologically. All of this adds to the complexity of flowering.

Descriptions of the anatomy of the flower or infloresence may be obtained for some genera from general taxonomy references (Lawrence 1951). Times of anthesis and seed maturity are generally given in silvics references (Fowells 1965).

Acer (maples)

Acer is reproductively very diverse even within a species. This is demonstrated by A. pensylvanicum in which individuals are generally dioecious although monoecious individuals occur (de Jong 1976, Hibbs and Fischer 1979); inflorescences generally contain only staminate (male) or pistillate (female) flowers but may contain both; flowers are imperfect or rarely perfect (although not functionally) (Hibbs and Fischer 1979), and the proportion of flower types in a tree may change from year to year. Buds are mixed in some species (Hibbs and Fischer 1979, Anderson and Guard 1964) or both mixed and simple in others (Barker et al. 1982). Some of this variation results from tree age (Hibbs and Fischer 1979) or environmental influence (Barker et al. 1982).

Floral initiation occurs by the transition of a vegetative apex to a floral apex in late June in A. pseudoplatanus but the time of initiation is not known for other species. Individual floral primordia are then initiated as lateral appendages along the main inflorescence axis (Anderson and Guard 1964). There are no references to possible variations in time of floral initiation or to stage of floral development present in overwintering buds. There is no evidence of different sex expression in buds from different regions of the crown (Hibbs and Fischer 1979, Barker et al. 1982).

Betula (birch)

Betula is monoecious. In B. papyrifera, vegetative short shoot, long shoot, and female and male inflorescence initiation and development have been carefully described (Caesar and MacDonald 1983, MacDonald and Mothersill 1983, MacDonald et al. 1984).

Female inflorescences form on short shoots. Short shoots develop from proximal axillary buds on long shoots (potential short shoot bud), from short shoot terminal buds or from axillary buds on flowering short shoots. Axillary short shoot buds are initiated in the summer and undergo a vegetative growth cycle. The vegetative cycle may be repeated for several years or the short shoot apex may undergo transition to a female inflorescence apex. The transition of axillary buds on a long shoot will occur after only one year of vegetative growth. Transition of a terminal bud may occur in any year. Transition occurs in late June. Before winter dormancy, inflorescence bracts develop and gynoecial (female) floral parts partially develop in their axils. Stigmatic primordia are not initiated until after winter dormancy.

Male inflorescences form on long shoots. Long shoot buds are initiated in the axils of distal late leaves of the elongating long shoot. The long shoot bud develops one rudimentary leaf, two or three embryonic leaves, and about five smaller primordia which expand the following spring into distal late leaves. Male inflorescences are initiated in early May (before flushing) as primordia in the axils of the last formed leaf primordium, and in the axil of a transitional leaf on the long shoot. Terminal apices may abort or form male inflorescences. Bracts form rapidly on the inflorescence apex and, by early June, floret primordia are initiated in bract axils. Male inflorescences are well developed before winter dormancy, are not enclosed by bud scales (naked), and are located at the apex of long shoots. Terminal male inflorescences impose limitations on crown expansion because they significantly reduce leaf area (Caesar and MacDonald 1984) and prevent shoot development (MacDonald et al. 1984). There is no clear separation of female and male bearing shoots on branches or within the crown.

Populus (poplars, aspens, cottonwoods)

Reproductive buds are simple (Jackson and Sweet 1972). Graf (1921) described differentiation of staminate and pistillate inflorescences. Nagaraj (1952) and Beetle (1974) described floral initiation. Although the genus is generally regarded as dioecious, the occurrence of monoecious inflorescences and perfect flowers has been reported (Lester 1963). Inflorescence buds of P. tremuloides may develop into pistillate, staminate, or perfect flowers, which are initiated at different times (Lester 1963). Inflorescence buds are initiated in the axils of leaves on the current year's growth. These axillary buds are small apices, each with one bud scale when winter dormancy occurs.

Terminal buds burst in May. Axillary primordia initiate several bud scales during rapid shoot elongation. Floral apices are determined about mid-June (Lester 1963, Beetle 1974). Pistillate flower primordia begin development of floral parts by late June and staminate flowers begin development of floral parts in early July. In perfect flowers, stamens are borne on the sides of the pistil primordia and the latter are initiated slightly earlier. Perfect flowers are restricted to the distal half of the inflorescence. Floral development continues through September, so that anthers and ovules are well developed in staminate, pistillate, and perfect flowers before winter dormancy. Brief descriptions of floral initiation and early development in P. tremuloides and P. deltoides (Nagaraj 1952) indicate similar phenology to that described by Lester (1963) and Seitz (1958). There are no descriptions of the distribution of floral buds in the crown or on branches.

Quercus (oaks)

Quercus is monoecious and flowers are imperfect (McDonald 1969). Buds are mixed, containing both leaves and flowers. Inflorescences occur in the axils of leaves and bracts, and terminal apices remain vegetative (Jackson and Sweet 1972). Only in Q. alba has floral development been studied in detail (Merkle et al. 1980). Both staminate and pistillate inflorescences may occur within the same bud. In May, staminate inflorescences are initiated in the axils of developing leaf primordia within vegetative buds. The half-cylindrical shape of the catkin quickly becomes evident and staminate flowers begin to develop acropetally in late June or early July. By early August, the catkin primordia are distinguishable microscopically and they are well developed when they become dormant in October. Pistillate inflorescence primordia are initiated in axils of the distal three or four leaves of the bud in July, but do not become distinguishable from lateral vegetative buds until August. One or two bracts are then formed but these develop little before winter dormancy begins in October. Pistillate floral development resumes the following March or early April, and is completed within three weeks (Merkle et al. 1980).

Similar floral initiation times were also reported for Q. alba (Turkel et al. 1955) and Q. robur (Lohwag 1910, Romasov 1957). However, the stage to which pistillate inflorescences develop in the fall may be quite variable among locations or species. There are no descriptions of the distribution of floral buds in the crown or on branches.

Other hardwoods

Several reports on other hardwood forest species show that flowering is affected by environmental and cultural factors occurring in the late spring and summer of the year before anthesis. These include Fagus spp. (Matthews 1955, Holmsgaard and Olson 1960, 1961, 1966, Matyas 1969), Fraxinus spp. (Pond 1936), Salix spp. (Junttila 1980), and Juglans spp. (Ponder 1979). These effects suggest the time of floral bud determination. However, such indirect evidence should be verified by developmental studies to determine if floral initiation, or a subsequent stage of development, is being influenced. Most studies of woody angiosperms do not make a clear distinction between floral initiation and floral development. The term "initiation" has been used too loosely in the literature on hardwood forest trees. The literature on fruit trees is less ambiguous and confirms that floral initiation, and at least the early stages of floral development frequently occurs in late spring or summer of the year before anthesis (Matthews 1963).

Summary, and recommendations for future research

Knowing the time and method of floral initiation is valuable both in determining environmental or developmental factors which may affect floral initiation, and in determining the correct time to attempt floral enhancement or induction treatments. There is no single factor responsible for floral initiation. The generalization that it occurs during the spring or summer before pollination is true for most forest trees; however, there are exceptions in the conifers (eg. Pinus) and perhaps in hardwoods. Conifers have simple buds and unisexual flowers. Hardwoods have either simple or mixed buds and often have hermaphroditic flowers. The complexity of flowering in hardwoods, along with lesser degree of commercial interest, has resulted in much less knowledge of floral initiation in hardwoods than in conifers. We should not assume that undescribed species resemble related species, especially in the hardwoods, which are quite variable.

Future research should emphasize those genera and species which have not been studied. An understanding of floral initiation is an essential first step in any floral induction project. The small early investment can yield considerable savings in time and money when experiments begin. It is impossible to evaluate a floral induction technique or compare results from different laboratories if the time of floral initiation is ignored. This becomes apparent when trying to evaluate the early literature on environmental effects on floral initiation (Ch. 3) and floral induction (Ch. 4).

The time and method of floral initiation should be determined for any conifer or hardwood species included in a genetic tree improvement or seed orchard programme. Time of floral initiation, determined anatomically, should be correlated with some easily observed developmental stage. Per cent of vegetative shoot elongation has been used for this purpose in several conifers. Calendar data can be used as an estimate for trees growing within their natural range, but this may not be accurate for trees grown outside of their natural range or in pots in growth facilities. Not all species require detailed anatomical study. Buds dissected during the growing season will often give enough information.

Floral initiation must be distinguished from subsequent floral development. Factors affecting floral initiation may have no effect on floral development and vice versa. We must know if we are affecting the occurrence of potentially reproductive apices, the differentiation of the apices into floral apices, reducing abortion, or affecting subsequent floral development. Without this knowledge there can be an endless proliferation of studies with inconclusive, conflicting, and unrepeatable results.

The time between biochemical differentiation and anatomical differentiation is not known for any forest species. Antigen antibody techniques now being used in animal and, to a lesser extent, in plant developmental studies could be adapted and used for the early detection of subtle molecular changes in apices long before morphological differences appear. This approach has been used in angiosperms (Pierard et al. 1977, 1980), and could aid biochemical and physiological studies as well as provide precise timing of floral induction treatments.

Finally, terminology should be standardized as much as possible to avoid confusion and allow easier comparison of results. The first changes during floral initiation are remarkably similar in conifers and hardwoods because they involve the transition of an apex undergoing indeterminate growth into an apex with determined growth. Therefore, the same terminology may apply to both. However, subsequent development becomes quite different because of basic structural differences between strobili, and flowers or inflorescences.

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CHAPTER 3

ENVIRONMENTAL FACTORS AFFECTING FLORAL INITIATION

Introduction

The preceding chapters demonstrate that all reproductive shoots of conifers and hardwoods differentiate from apices that are undetermined or were previously vegetative but pass through a very plastic phase in development. In most species this phase lasts several weeks and occurs during the year before anthesis. At this time environmental and endogenous factors interact to control bud determination. Many studies have tried to demonstrate a relationship between environmental factors and floral initiation in geographic regions, stands, and on individual trees but such relationships are frequently difficult to identify. For example, a correlation between seed crop and weather data is probable. However, Rehfeldt et al. (1971) cautions that, "...in any attempt to correlate previous cone crops with previous weather records, problems arising from intercorrelations among the dependent and independent variables will be encountered; it is difficult to identify causal mechanisms because of these intercorrelations." Similar intercorrelations may occur between, or among all factors affecting floral initiation and they should be considered in all attempts to interpret the effects of environmental factors. Reviews by Matthews (1963), Jackson and Sweet (1972), Puritch (1972), Lee (1979), Lavender and Zaerr (1985), Lavender (1985), and Pharis and Ross (1985) should be consulted because each provides a different perspective.

Periodicity

Long term fluctuations or periodicities in the production of cone and fruit crops have been demonstrated in conifers (Daubenmire 1960, Matthews 1962, Lowry 1966, Eis 1967, Van Vredenburch and La Bastide 1969, Zasada and Viereck 1970. Fober 1976) and hardwoods (Matthews 1955, 1963, Holmsgaard and Olsen 1961, Matyas 1969, Grisez 1975). Some genera, like Acer, Fagus, Betula, and Fraxinus (Holmsgaard 1972, Zasada and Gregory 1972, Grisez 1975) produce seed almost every year; others, like apple (Fulford 1960), are primarily biennial and produce good seed crops only every few years (see Fowells 1965). Some genera contain consistently good seed producers, e.g. Quercus rubra, and other species that may be good or poor seed producers, e.g. Q. alba (Grisez 1975). However, cone, fruit, or seed production represents the climax of the entire reproductive cycle and that can be influenced by many factors. Production records are generally compiled for stands and may obscure periodicity within individual trees. Periodicity data should be cautiously interpreted when related to specific environmental factors.

Temperature

A certain minimum degree of heat is required for floral initiation and this appears to be higher than that required for vegetative growth (Matthews 1963). High summer temperatures favor increased floral initiation and development. This has been demonstrated in several genera. The concept was first mentioned by Linnaeus (1751 from Holmsgaard 1972) who noticed that heat (and drought) in 1748 caused more abundant flowering than usual in Fagus in 1749. In Fagus (Matthews 1955, Holmsgaard and Olson 1960, Matyas 1969, Holmsgaard 1972), warm temperatures in June and July enhanced flower production, especially male, whereas cool autumns stimulated female flowering. Unfortunately, the time and method of floral initiation have not been determined for Fagus.

The effect of temperature on flowering has not been studied in other hardwood genera. Indirect evidence suggests that higher than normal temperatures may enhance flowering in young Betula, but these were grown under continuous long days (Longman 1976). Enhanced flowering in Betula grown in shelter houses is used in Scandinavian breeding programs. The time of floral initiation in some hardwoods is long, making it possible for temperature to have an effect on flowering and sex of induced flowers. In some flowering citrus trees, high summer temperatures are inhibitory to floral initiation (Jackson and Sweet 1972).

A positive correlation between high summer temperatures and floral initiation has been demonstrated in several conifers. Maguire (1956) used a 23-year record, and Daubenmire (1960) a 7-year record, of temperature and cone production for Pinus ponderosa. Maguire's results demonstrated that above average temperatures in April and May, in the year of reproductive bud determination, led to good cone crops 27 months later. These results are difficult to reconcile with the observations that seed cone bud differentiation in hard pines occurs in late summer and fall. In contrast, Daubenmire found no effect of April-May temperatures but a good correlation between higher than average temperatures in June to September and cone production.

Other studies of Pinus are less complete and often do not separate high light intensity and high temperature. Hagem (1917) and Fober (1976) suggest that high summer temperatures are necessary in the year of reproductive bud differentiation for good cone production in P. sylvestris. Fober (1976) also found that warm weather in April, July, and August of the year before reproductive bud differentiation was important and he attributed this to an accumulation of storage substances that increase tree vigor. High mean July temperatures correlated positively with seed cone production (based on one year old conelets) in P. resinosa (Lester 1963). In a review on Pinus, Lee (1979) concluded that high temperatures throughout the growing season will enhance reproductive bud initiation. There are no references relating temperature differentiation in the soft pines (haploxylon).

In Abies, Picea, and Pseudotsuga, which have similar times and methods of reproductive bud differentiation, there is a good correlation between higher than average summer temperatures at the time of differentiation and good cone crops the next year. Eis (1973) based his conclusion on 36 year records of A. grandis. Other studies show similar results for Picea (Fraser 1958, Lindgren et al. 1977) and Larix (Yanagihara et al. 1960). The most complete studies have been of Pseudotsuga (Lowry 1966, Van Vredenburch and La Bastide 1969, Eis 1973). In addition to warm temperature at the time of reproductive bud differentiation, they also found positive correlations between good cone crops and cool, cloudy weather the summer before bud differentiation. This covers the period of vegetative bud development when leaf primordia are initiated. Potential reproductive bud apices are initiated the following spring within the bud in the axils of these leaves (Owens 1969). Vigorous vegetative growth could lead to the initiation of more leaf primordia, providing more sites for axillary bud initiation the following year.

High temperatures during reproductive bud differentiation may affect various metabolic processes within the plant, including photosynthesis, carbohydrate metabolism, water and nutrient uptake, transpiration, and growth substances. Unfortunately, we know little about the effect of temperature on these processes in reproductively mature trees. The problem becomes more complex when an attempt is made to separate the effects of increased temperature from the effects of light intensity and moisture, since cloudy, wet days are often associated with low temperatures.

Light intensity

Most studies of effects of light intensity on flowering are indirect. They relate flowering to crown exposure, slope, floral distribution within the crown, and shading. Generally, branches exposed to high light intensity tend to flower more than shaded branches. Nanda (1962) found flowering in closed stands of Tectonia to be confined to upper parts of crowns of dominant and co-dominant trees exposed to bright sunlight. Crown closure in Acer pensylvanicum altered light intensity and caused a change in sex of flowers produced. In open crowns most flowers are male but as crown closure occurs there is a decrease in male and an increase in female flowers. This increases the seed productivity of the stand with time (Hibbs and Fischer 1979). Thinning has been a successful method of floral induction in Pinus (Phares and Rogers 1962, Halls and Hawley 1954, Barnes 1969, Allen 1953, Godman 1962, Cooley 1970, Eldridge 1966, Wenger 1954, Bilan 1960, Allen and Trousdell 1961) and Pseudotsuga (Reukema 1961).

Sarvas (1962) observed that flowering was most abundant on exposed P. sylvestris trees and that light intensity may affect development of reproductive structures. Brøndo (1970) and Simpson and Powell (1981) showed that flowering was more abundant in trees growing on south slopes than on other aspects. Similarly, flowering is commonly most abundant on the south side of the crown (Winjun and Johnson 1964, Smith and Stanley 1969). Shading experiments of individual branches decreased seed cone bud production in Pseudotsuga (Silen 1973), Cryptomeria (Migita 1960a, b), and certain fruit trees (Jackson and Sweet 1972).

Although the reasons why increased light intensity may increase flowering on entire trees or separate branches are not clear, results indicate that it may be a practical method of enhancing floral initiation under certain conditions. It could also be an important consideration in the selection and management of tree crown form for maximum seed production.

Photoperiod

Photoperiod, the length of the relative light and dark periods, has not been demonstrated to have a direct effect on forest trees as it does in many herbaceous plants. Mirov (1956) concluded from his study of 28 species of Pinus that they were day neutral, that is, flowering was not affected by daylength. Wareing (1958) also concluded that photoperiod had no effect on flowering in P. sylvestris, as did Lanner (1963) for Pinus. A negative effect of photoperiod using interrupted nights was observed for P. attenuata (Lanner 1963) and Picea glauca (Durzan and Campbell 1979). Dunburg (1979) suggested that photoperiod may be more important in development than light intensity. He presented evidence that effects of latitudinal movement on floral induction in Picea may be photoperiodic.

There is some evidence that photoperiod may affect the sex of reproductive buds. Longman (1961) found that potted young Pinus contorta grown under short days out-of-doors formed more seed cones than controls grown under natural daylengths. This could have resulted primarily from a decrease in shoot elongation caused by short days (Greenwood 1981). Giertych (1967) proposed that pollen cone buds, which are initiated during longer daylengths than seed cone buds in Pinus, constitute an example of photoperiodic effects on flowering in conifers. Differences in natural time of reproductive bud differentiation in Thuja plicata led Owens and Pharis (1971) to propose that, although differences in actual daylength may be too slight for the tree to perceive, the difference between increasing (pollen cone initiation) and decreasing (seed cone initiation) daylengths may not be.

Studies of Tsuga heterophylla and Chamaecyparis nootkatensis (Owens and Molder 1974a, b) also showed that pollen cone buds were initiated under increasing daylengths and seed cone buds under decreasing daylengths. Pharis and Morf (1967) reported precocious flowering in four-month-old seedlings of Thuga plicata treated with gibberellins. Seedlings kept under long days (16 h of light), formed over six times more reproductive buds as under short days. In C. nootkatensis seedlings in which reproductive buds were induced with gibberellin A_3 (GA_3), a higher proportion of pollen cones were produced under long days (16 h of light) than under short days (8 h of light) (Owens and Molder 1977f). A long day requirement was also demonstrated in GA_3-induced pollen cones in Cupressus arizonica (Pharis et al. 1970). In all conifers where there is a demonstrated effect of photoperiod on bud induction or sex, there is a natural difference in time of male and female cone bud differentiation. In species without this difference, photoperiod may act indirectly by causing the end of shoot elongation which coincides, in conifers studied thus far, with reproductive bud differentiation (Owens 1980, Owens et al. 1985).

In woody angiosperms, photoperiod appears to control cessation of shoot elongation which often coincides with the time of floral initiation (Jackson and Sweet 1972). Thus, photoperiod may influence floral initiation and sex of flowers, especially in those species where male and female flowers become determined at different times. As in conifers, it may be difficult to separate effects of photoperiod and light intensity. There are no direct photoperiodic effects on floral initiation reported for hardwood forest trees.

Moisture

Under natural conditions the effects of moisture are difficult to separate from other factors. Low moisture availability is frequently associated with increased reproductive bud initiation but low moisture usually accompanies high temperatures and high light intensities. Several studies have shown a positive correlation between increased flowering and possible low moisture availability in the spring and summer of the previous year, usually resulting from low rainfall. In Fagus sylvatica, dry summers (and high temperatures) were correlated with increased seed production over a 100-year period (Holmsgaard and Olsen 1960). However, Matthews (1955) found that regressions of size of seed and rainfall were not significant. Since then, experiments on beech seed production have demonstrated an effect of water supply separate from temperature (Holmsgaard and Olsen 1961, 1966, Holmsgaard 1972). Holmsgaard and Olson (1966) subjected potted beech grafts to drought at different times and found that the latest and longest treatment in the summer produced the largest seed crop. Beyond the reviews by Matthews (1963) and Jackson and Sweet (1972) the more recent literature provides no further information on temperature effects on flowering in hardwood forest trees.

Conifers have been studied more extensively. Studies have generally shown positive correlations between low rainfall during the spring or summer when reproductive buds become determined, and subsequent cone production. Unfortunately, the precise time of reproductive bud determination was not known or considered when many of these studies were done. This has led to some conflicting results. Also, the results of many experiments using water stress are confounded by other treatments.

Studies of Pinus monticola compared yearly counts of seed cones at various stages of development with analysis of daily means of moisture stress (Zahner and Stage 1966) over an 18-year period (Rehfeldt et al. 1971). They found that high moisture stress during early summer, two years before cone emergence, was associated with high cone counts. However, high water deficits during late summer of the year preceding cone emergence were detrimental to cone production. The latter stage is when potential seed cone and lateral long shoot buds are initiated (but not differentiated) in soft pines (Owens and Molder 1977c). Unfortunately, water deficits were only calculated from June through September (Rehfeldt et al. 1971), and not in early spring, the period of seed cone bud differentiation in soft pines (Owens and Molder 1977c).

In P. palustris, a hard pine, high spring rainfall enhanced seed cone bud formation if early summer rainfall was also high in the year of reproductive bud differentiation. Flowering always increased with higher rainfall in June. It also increased with July rainfall unless the spring was very dry (Shoulders 1967). These data are difficult to interpret because of simultaneous fertilizer treatments and because they do not include the later summer and fall treatments when seed cone bud determination occurs in hard pines (Owens and Molder 1975a). Fober (1976) concluded from a correlation of meteorological factors and seed crops in P. sylvestris that seed cone bud initiation was most abundant during years when there was a sunny, dry spring (March and April) and a warm, sunny summer (July). Close analysis of these data show no correlation between precipitation alone and seed cone production during the late summer period when seed cone buds differentiate in hard pines. Therefore, it is difficult to generalize about moisture effects on seed cone initiation in either hard or soft pine.

In Picea abies warm and dry summers usually enhance seed cone bud production (La Bastide and Van Vredenburch 1970, Eriksson et al. 1975, Lindgren et al. 1977). However, Sarvas (1957) studied meteorological data from a 55-year period and found many years when there was a high incidence of high summer temperatures and summer droughts that were not followed by abundant cone crops on P. abies. He concluded that the temperature/drought factor was not a decisive, but rather a contributing, factor in seed cone bud determination. In Abies grandis and Pseudotsuga menziesii a wet April, 16 months before cone maturation (during early elongation of the shoot on which seed cone buds develop), and a warm, dry, sunny June (during early stages of reproductive bud differentiation) resulted in a higher proportion of seed cone than pollen cone buds (Eis 1973). Lowry (1966) also found a positive correlation between high spring precipitation (two to three months before reproductive cone bud differentiation) and increased seed cone production in P. menziesii, but no correlation with a dry summer during seed cone bud determination. Van Vredenburch and La Bastide (1969) showed a correlation between increased seed cone production and sunny, warm, and dry weather during June and July of the year of bud differentiation in P. menziesii. Fraser (1958) reported that, in natural stands, water stress on drier sites was sufficient to reduce radial growth but did not affect seed cone production in Picea glauca. From this he concluded that moisture was of secondary importance in flowering.

When precipitation alone is considered, data generally do not show consistent positive correlations between low precipitation at the time of reproductive bud differentiation and enhanced floral production. Some positive correlations, which occur before bud determination, may result from metabolic processes which enhance initiation of undetermined buds, promote their early development, or predispose them to become reproductive buds. More conclusive data on how moisture affects floral initiation comes from experiments in which water availability to the tree has been controlled and moisture stress measured. These experiments show a definite promotive effect of withholding water and subsequent moisture stress on floral production. This will be discussed in Chapter 4.

Mineral nutrients

Generally, there have been few studies devoted to mineral nutrients but occasionally observations are included as parts of other studies. All other factors being equal, trees growing on fertile sites tend to produce more seed than those on less fertile sites (Matthews 1963). Unfortunately, few studies of nutrient levels discriminate between effects on floral initiation, seed production, and seed quality. Nemec (1956) showed that nut formation in Fagus sylvatica involved considerable consumption of mineral nutrients, and that a decline in mineral content of soil, accompanied by acidification of the soil profile, was associated with poor or no seed production. In Pinus sylvestris in Finland, flowering and seed production were better on more fertile sites (Sarvas 1962). In that report, pollen cone production on fertile sites was four times that of trees growing on infertile sites. Crocker (1973) reported that the seed crop of P. palustris was enhanced by increasing site fertility. Site fertility may be affected by ground vegetation. Azniev (1970) observed that in P. sylvestris plantations, the presence of lupin caused earlier and heavier cone production.

Mineral nutrients that appear to be most important in flowering are nitrogen (N) and phosphorus (P) (see review by Jackson and Sweet 1972). Their specific role, if any, in flowering is not clear. Most information comes from experiments where application of P and especially N may stimulate flowering but the form of N applied and the time of application are also important. These aspects will be discussed in Chapter 4.

Effects of other mineral nutrients are not as well documented. Reduced floral initiation has been reported with copper deficiency in apples and pears (Wallace 1961) and a positive correlation has been shown between calcium content of Fraxinus and apple shoots and flower bud formation (Perfil'ev 1962). Deficiencies of calcium and magnesium may reduce flowering in Cryptomeria (Lyr and Hoffman 1964). It is likely that many nutrient deficiencies may adversely affect flowering and seed production.

Other factors

There are several factors which through injury or stress may affect floral initiation positively or negatively. After injury, trees often respond by increased sexual reproduction, referred to as stress crops (Brøndo 1970). Injury, leading to stress crops, may be inflicted in a variety of ways.

Late frost damage has been reported to increase Larix cone crops the following year (Wachter 1959, 1962). Ebell (1971) reported increased cone production in Pseudotsuga menziesii following frost damage in November which caused lesions that girdled the trees to varying degrees. Any form of girdling may induce a stress crop, supposedly by hindering downward movement of carbohydrate in the phloem. This often results in enhanced reproductive bud differentiation the following year. Wounds resulting in some degree of girdling may result from various causes, including logging, fire, insects, mammals, and disease. Girdling used as a cultural treatment to induce flowering will be discussed in Chapter 4.

Resin tapping has been reported to decrease cone production in *Pinus sylvestris* (Vornov 1962) and also increase it (Philippis et al. 1966). In the latter case, the initial increase, perhaps resulting from partial girdling, was followed by a decrease in cone production.

Defoliation, if done before early May, increased the initiation of pollen cones in *P. sylvestris* but had no effect on branch development (Giertych 1970). He suggested reduced shoot vigor favored pollen cone bud differentiation. There are reports relating natural defoliation (by insects) to increased cone production. Top dying has increased seed production in *Betula* (Kessler 1969).

Root damage by logging, road building, excavation, and disease may enhance reproductive bud initiation. This has been reported in *Sequoia sempervirens* (Muelder and Hansen 1961) and *Picea abies* (Starcenko 1964), and the occurrence is very common and familiar to many foresters. The effect of root damage may be through reduced water uptake resulting in a moisture stress within the tree. Moisture stress through rootpruning as a cultural treatment for seed cone induction will be discussed in Chapter 4.

Stress crops may seem counter productive for an individual tree. The diversion of a limited supply of energy into reproductive structures may result in further stress leading to death of the tree. However, the phenomenon has definite advantages for the survival of a species.

Influence of endogenous cycles

The lack of consistent positive correlations between environmental factors and seed production often results from the failure of cone, fruit, or seed development despite increases in reproductive bud differentiation. Another consideration is the endogenous cycle within a tree. Although we do not understand all of the physiological reasons for reproductive bud differentiation, we do know that in most fruit and forest trees abundant fruit and cone crops do not occur on the same tree in consecutive years. An abundant crop is usually followed by no crop or a very small crop. This is because, in most species, the time of reproductive bud differentiation coincides with the most rapid growth of the fruit or cones which have high nutrient requirements (Kozlowski and Keller 1966). Consequently, shoot elongation and reproductive bud development on these shoots is inhibited by subtending fruits and cones (Allen and Owens 1972, Powell 1977a, Owens 1984b).

Considerable energy goes into reproductive growth. In mature *Pinus radiata*, the pollen, cones, and seeds make up 16 per cent of total dry weight of the tree (Fielding 1960). Appreciable quantities of minerals and nitrogen also accumulate in fruits and seeds (McKee 1958, Bell and Childers 1954, Dickman and Kozlowski 1969a, b). In *P. resinosa* (Dickman and Kozlowski 1970), translocation and incorporation of ^{14}C-labelled photosynthate was taken up initially by second year cones followed, in order, by terminal shoot needles, lateral shoot needles, terminal shoot internodes, lateral shoot internodes, and one year old wood. Reproductive buds of *Pinus* differentiate from axillary apices in internodal regions of terminal and lateral long shoot buds, which are not strong sinks for assimilates. In other members of the Pinaceae as well, the subtending shoots (Loach and Little 1973) and seed cones (Ching and Ching 1962) are primary sinks for photosynthate at the expense of elongating shoots on which reproductive buds differentiate. The effect of developing fruits and cones on growth regulators in elongating shoots and developing buds is not known.

It appears to take one or more years following a heavy crop, for trees to recover. No matter how favorable environmental factors might be, they rarely override the effects of bearing a heavy crop and consecutive crops do not occur. Therefore, although environmental factors may be ideal, correlation with a good crop may not occur. Also, after a tree recovers from a heavy crop, less than optimal environmental factors might enhance a moderate to heavy crop. Another factor which might affect correlations within a stand or region is the chance synchronization of many trees, each with its own crop periodicity. This, coupled with favorable environmental factors, could result in the bumper crops which occasionally occur in many species within a region in one year. No doubt environmental factors play an important role, but definitive studies of their effects may never be possible because of the multitude of as yet poorly understood interactions between exogenous and endogenous factors.

Summary, and recommendations for future research

Most studies of environmental effects on forest tree reproduction have been done without knowledge of the natural time of floral initiation or a clear distinction between factors affecting bud initiation, bud differentiation, and subsequent development through all of the stages to cone or fruit maturity. It is not surprising that many apparent correlations cannot be explained developmentally or physiologically and many expected correlations do not occur. The reproductive cycles, which usually take two or more years from floral initiation until seed release, do not lend themselves to this sort of analysis. Nevertheless, some generalizations can be made regarding factors affecting floral initiation. It is generally favored by high light intensity and temperatures, low rainfall and soil moisture, and high soil fertility at, or preceding, the time of bud determination. Many natural phenomena causing stress within trees may also enhance flowering. Under natural conditions it is nearly impossible to separate the interaction of all factors. Added to the complex interactions of factors is the natural endogenous cycle within a tree and perhaps within a stand or region. Environmental factors may act to synchronize these cycles.

Further attempts to correlate flowering with environmental factors may be of marginal benefit. Despite rapid development of statistical techniques and computer capabilities there is still a general lack of good long term weather and crop data for a given region. We do not yet know how a single factor (eg. drought) affects the physiology of the tree and floral initiation. Therefore, we cannot hope to sort out the complex interactions between all factors. More useful research would be to study the effects of individual factors on the physiology and development of trees grown under controlled conditions. Small rooted cuttings rather than grafts or seedlings provide physiological clones suitable for experimental studies in controlled environments. Using these trees, researchers could study in detail the most important factors (e.g. temperature, light, drought) influencing reproductive development throughout the long reproductive cycles. This would be a large task, coordinating studies of development with changes in endogenous growth regulators, carbohydrates, and perhaps certain amino acids which are implicated in reproductive development, and subsequent interpretation of results. Analytical techniques requiring only small amounts of tissue are being developed. Researchers, equipment, techniques, and suitable plant material of several species are now available for this type of research.

CHAPTER 4

FLORAL INDUCTION AND ENHANCEMENT

Introduction

Floral induction in juvenile or otherwise non-reproductive trees, and the enhancement of flowering by cultural treatments, are valuable tools in genetic tree improvement programs and in seed production for reforestation. Most cultural treatments involve alteration of the environmental factors described in Chapter 3. Many treatments are not easily applied to large trees, nor are they easily controlled or repeatable under field conditions. Also, it is not possible to ensure that all field environmental conditions remain uniform while under study. Consequently, most cone induction or enhancement trials have been confounded by the interaction of several variables leading to inconclusive results. The use of containerized (potted), genetically uniform, small trees grown in controlled environments will provide better conditions for determining the relative importance of individual treatments and their interactions.

Fertilizer treatments

The application of nitrogenous fertilizers is one of the oldest and most widely used floral induction/enhancement treatments. Matthews (1963) reviewed early fertilizer treatments and discussed them in relation to the carbohydrate/nitrogen (C/N) ratio. A review of fertilizer experiments on conifers through 1970 is given by Puritch (1972). Jackson and Sweet (1972) also reviewed the literature on N and phosphorous (P) fertilizer treatments of fruit trees and hardwood and coniferous forest trees. In addition, they briefly discussed other mineral nutrients. An annotated bibliography on the effects of fertilizer on cone and seed production has been done by Brazeau and Veilleux (1976). More recently, Lee (1979) reviewed site fertility and fertilizer treatments on cone induction primarily in Pinus.

There have been several reports on fertilizer treatments since the extensive reviews by Puritch (1972) and Jackson and Sweet (1972) which have not been described in other reviews. Rather than discuss all of the reports in detail, Appendix 1 provides a list of species fertilized, the treatments used, times of treatment if specified, the effects on flowering and appropriate references. This includes all references from Puritch's (1972) review and more recent reports. Some general observations based on these treatments are given below.

Results from fertilizer treatments are extremely variable for several reasons. Fertilizers have been applied at different times of the year, often without careful regard for the natural time of floral initiation in a species. Unless fertilizer is applied long enough before floral initiation to allow for lag-time in uptake, results will likely be negligible. Several fertilizer treatments of Pseudotsuga applied during the period of vegetative bud break (Stoate et al. 1961, Ebell 1972b) resulted in enhanced cone production. Fertilization two weeks before or after flushing was ineffective. In Picea, spring (Holst 1971) and summer (Remrod 1972) treatments were best.

Fertilizer treatments have been applied at different times in several species of hard pines with variable results (Appendix 1). For example, Schmidtling (1974, 1975) increased female flowering in Pinus taeda with late summer application and attributed this to having applied the fertilizer during seed cone bud differentiation. Other fertilizer treatments showed increased flowering in pine, even though the time of treatment did not coincide with reproductive bud differentiation (Greenwood 1977, Heidmann et al. 1979). Results such as these have led Sweet and Hong (1978) to suggest that the major role of N, in increasing cone production in pines, may have been to increase crown size and number of sites in the crown where reproductive buds may be initiated.

There are few reports of fertilizer treatments of soft pines and increased flowering by fertilizer alone is not reported. A preliminary study reported increased cones in P. lambertiana with fertilizer treatment (Schubert 1956). The greatest increase in female flowering in P. monticola occurred when fertilizer was applied on April 30 of the year of anthesis as opposed to May 27 (Barnes and Bingham 1963). Earlier treatments may have better coincided with the time of seed cone bud differentiation (Owens and Molder 1977c). However, these treatments were confounded because they followed release treatments and increased watering given the preceding year. Fertilizer and high temperature treatments induced pollen and seed cone buds in P. strobus and one hybrid (Barnes and Bingham 1963).

Fertilizer treatments increased male and female flowers in beech (Fagus silvatica) in France and these effects were still present after three years (Le Tacon et al. 1977). This appears to have been a general response increasing tree vigor rather than a specific effect on floral initiation. There are no reports on effect of fertilizers specifically on floral initiation in hardwood forest trees. Jackson and Sweet (1972) describe fertilizer effects on floral initiation in several fruit trees.

In most fertilizer experiments the nutrient content of the forest soil was not analysed before treatment. Therefore, the nutrients which were deficient were not known and the types and amounts of nutrients added may or may not have been adequate. This could lead to extremely variable results in different species and sites. Seed cone crops may be increased just by increasing site fertility or index, as has been shown in P. sylvestris (Sarvas 1962) and P. palustris (Croker 1973).

The type of fertilizer is also important. In Pseudotsuga menziesii ammonium N did not induce reproductive buds, whereas nitrate N increased seed cone production up to seven (Ebell and McMullan 1970) and 10 (Ebell 1972a) times. Despite this effect, there was no difference in the accumulation of total N in buds and foliage (Ebell 1972a). Ammonium N increased total protein N, whereas nitrate N increased amino acid levels, particularly arginine and lysine. Ebell (1970) concluded that the different responses did not result from availability or rate of uptake of the two forms of N or from improved mineral nutrition, but from specific chemical stimulation of critically timed changes in the type of N metabolism. Barnes and Bengston (1968) found that fertilizing with NH_4NO_3 doubled seed cone bud initiation and increased free arginine, total free amino acids, and total N of twigs and total N of leaves in P. elliotti. It is evident from the many references (Appendix 1) that the form of N required for best results may vary with the species. Also, in Pseudotsuga, fertilizer application enhanced cone initiation only in naturally good years for cone initiation and was relatively ineffective in poor years (Steinbrenner et al. 1960, Ebell 1972b). In many studies the form of N is not specified. Other minerals, such as phosphorous (P) and potassium (K), are frequently applied with N making it difficult to determine their specific effect. Ebell (1962) got no response with P and K on Pseudotsuga but Giertych (1973) reported increased cone production in Pinus sylvestris with K fertilization. Adequate levels of P may be necessary for male flowering in P. radiata (Sweet and Will 1965) and Cryptomeria (Lyr and Hoffman 1964). Jackson and Sweet (1972) concluded that it was difficult to comprehend the role of P in the flowering of fruit trees.

The sex of initiated cones may be affected by fertilizer treatment. Lee (1979) suggests that the more frequent seed cone, as opposed to pollen cone, response may indicate different requirements of N nutrition in sex determination. However, most of his examples are for Pinus where pollen and seed cone buds are initiated at different times. Shoot vigor (Varnel 1976), exposure of crown (Sarvas 1962), and mineral deficiences (Sweet and Will 1965) have also been associated with the sex of cones. Consequently, there is no clear indication that fertilizer treatment alone can affect sexuality of cones.

Moisture and other environmental factors may alter the fertilizer effect. Rainfall after fertilizer application may negate the beneficial effects on cone production (Ebell 1972b). Many interactions have been shown between fertilizer, root pruning, and other water stress treatments.

Girdling, banding, and strangulation

These wound treatments have long been used successfully to induce flowering in fruit trees (see Holmes and Matthews 1951), conifers (Appendix 2), and at least one hardwood (Pond 1936). The intent of these treatments is to increase carbohydrate concentration in the crown by impeding its downward movement. This is a consequence of Klebs' (1910) original concept that conditions which favor carbon assimilation and limit uptake of soil nutrients enhance flowering. The carbon/nitrogen (C/N) ratio theory of Kraus and Kraybill (Kraus 1925) proposed that a high C/N ratio in the plant (high C) promotes flowering and a low ratio (high N) favors vegetative growth. A few experiments support this hypothesis but many others do not.

Girdling of *Cryptomeria japonica* and *Larix leptolepis* increased carbohydrates and decreased both water and N in shoots, resulting in a sharp increase in the C/N ratio and an increase in flowering (Hashizume 1970). Others doubt the significance of a high C/N ratio in flowering and consider it to be a consequence rather than a cause of flowering (Kramer and Kozlowski 1960, Ebell 1971). Unfortunately, with few exceptions (Hashizume 1970, Ebell 1971), most experiments report only the effect on flowering and do not relate this to actual changes in carbohydrates. It is difficult to reconcile the C/N ratio theory when N fertilizers can markedly enhance flowering and increase various forms of N within the tissues. Despite the lack of a satisfactory explanation for increased flowering by girdling and strangulation, both treatments can be successfully used for floral induction.

Results vary depending upon the time of application, the method of girdling or strangulation used, and the use of adjunct treatments. The most definitive results were based on experiments using double-stemmed *Pseudotsuga menziesii* where the second stem served as a control. Other trees were girdled at weekly intervals from April to mid-July to determine the optimal treatment time (Ebell 1971). Enhanced cone crops were obtained on girdled, as opposed to ungirdled, paired stems and the optimal time of girdling was about one month before vegetative buds burst. This is several weeks before the anatomical differentiation of buds (Owens 1969), but would allow adequate lag time for the treatment to be effective. Melchior (1960) found that girdling *L. leptolepis* up to the end of May enhanced flowering the following year, whereas girdling after June enhanced flowering two years later. He correctly reasoned that these results were because cones were initiated in June. In *L. occidentalis*, reproductive bud differentiation occurs in mid-June (Owens and Molder 1979b). Many other girdling and strangulation trails have been less precise in timing and have been assessed only by mature cone counts.

The method of girdling (Cade and Hsin 1977, Shearer and Schmidt 1970) and strangulation (banding) have variable effects on cone induction and tree damage. Thin (2.5 cm) overlapping girdles give good results and heal well, but repeated treatments can be harmful if the trees are to be used for long term cone production. Melchior (1961b) found the presence of branches below the point of girdling was necessary for survival of 6-year old *Larix* grafts. Ebell (1971) recommended girdling as a supplemental technique where response to other treatments has been poor. Branch girdling has been used successfully (Stephens 1964) and offers an alternative where limited cone production is required.

Strangulation by various techniques provides a temporary and severe restriction of translocation but results are generally less effective than from girdling. The poor response to strangulation may result from the incomplete, slower, and therefore poorly timed restriction of translocation (Ebell 1971). Flowering has been enhanced by strangulation in several conifers and in *Fraxinus nigra* (Pond 1936). However, many negative results are also reported (Appendix 2) and Bilan (1960) reported adverse effects through starvation of roots.

Moisture Stress

The effect of natural moisture stress on floral induction has been known for many years and has led to many attempts to induce flowering by various cultural treatments (Appendices 3 and 4). These include controlled irrigation, drought treatments, and rootpruning. Not only has withholding water stimulated flowering but, in some pines, watering from March to November increased the number of pollen cones produced the next season (Barnes and Bingham 1963, Barnes and Bengston 1968, Bengston 1969, Dewers and Moehring 1970). Others have demonstrated increased flowering in pines when irrigation was combined with other treatments such as disking (Schultz 1971), fertilizer (Sprague et al. 1979), and subsequent drought (Dewers and Moehring 1970).

Ebell (1970) demonstrated in *P. menziesii* that moisture stress increased the level of amino acids, particularly arginine, and induced cones (as did nitrate N), whereas readily available water increased protein levels but did not induce cones (as did ammonium N) (Ch. 2).

Carefully controlled drought treatments are difficult to perform in the field and many attempts have not been accompanied by water potential measurements. Consequently, field drought treatments can give highly variable results. Experiments using containerized trees and carefully monitored water potential show that moisture stress does enhance flowering in some species. Pre-dawn moisture stresses of 12 to 20 atmospheres, measured by the pressure chamber technique (Ritchie and Hinkley 1975), appear minimal for cone induction. Unstressed trees in the field can be expected to average about 7 to 8 atmospheres of moisture stress (Cade and Jackson 1976). Consequently, many unmeasured moisture stress conditions which have not resulted in cone induction may represent trees under low moisture stress. Potted *Fagus* grafts subjected to drought for varying periods from May 27 to June 27 showed enhanced flowering from the later treatments (Holmsgaard and Olsen 1966). Flowering in potted grafts and seedlings of *P. menziesii* was stimulated by drought treatments severe enough to halve shoot elongation (Ebell 1970). Treatment beginning at the time of flushing and continuing for the next three weeks was optimal. Results from drought treatments of containerized trees may be confounded by heat effects if experiments are done indoors.

Moisture stress in field grown trees is often brought about by rootpruning. Rootpruning increased seed cone initiation in *Pinus elliottii* (Hoekstra and Mergen 1957) and *P. strobus* (Stephens 1961, 1964), but had no effect on *L. leptolepis* (Heitmuller and Melchior 1960) and *Pseudotsuga menziesii* (Melchior 1968). March rootpruning of *P. menziesii* resulted in reduced water potential in June (when reproductive buds differentiate), reduced shoot elongation (Webber et al. 1985), and enhanced seed-cone initiation (Ross et al. 1985). Rootpruning also reduced mitotic activity in terminal and in potentially reproductive axillary buds. This delayed bud development and the time of bud determination by two to four weeks (Owens et al. 1985) and increased the proportion of aborted and latent buds (unpublished data). Delayed development moved the time of bud differentiation into July when endogenous and exogenous factors may have been more favorable for reproductive development (Owens et al. 1985).

Rootpruning may affect more than water uptake. In *Ribes*, roots produce a flower inhibiting substance (Schwabe and Al-Doori 1973) which may be removed with rootpruning (Dunberg 1977, Bonnet-Masimbert et al. 1982, Philipson 1983). However, similar flower inhibiting substances have not been demonstrated in forest trees. The cycle of root development is very complex and different from that of the shoot (Johnson-Flanagan and Owens 1985); therefore, rootpruning and drought should not be regarded simply as methods causing moisture stress.

Lifting and transplanting have enhanced cone initiation and may be comparable to rootpruning. Quirk (1973) lifted and pruned the roots, then replanted 10-year-old *Pinus resinosa* in April. These trees produced increased numbers of cones in the second and third growing season after this treatment while unlifted trees produced no cones. Silen (1973) moved seed orchard *Pseudotsuga menziesii* ramets and induced an average of 23 cones compared to zero for unmoved ramets. Moved ramets also showed drought symptoms.

Release

The effect of thinning on flowering in forest stands was discussed in Chapter 3 under light intensity. This form of release has been used to increase nut production in *Juglans* (Ponder 1979), flowering in other hardwoods (Kashimura et al. 1953), and cone production in several conifers (Appendix 5). The possible causes for increased reproduction are discussed in Chapter 3. Release is often accompanied by fertilizer treatment (Heidmann et al. 1979). Results can be large, such as the six fold increase in the number of cones obtained in *Pinus taeda* (Bilan 1960), and beneficial in both good and poor years (Wenger 1954, Allen and Trousdell 1961). In *P. resinosa* the per cent of trees bearing cones was directly proportional to the degree of thinning (Godman 1962). However, Florence and McWilliam (1956) demonstrated in stands of *P. elliottii* and *Araucaria cunninghamii* that the density giving maximum cone production per tree was much lower than the density giving maximum cone production per acre. From this they recommended optimal spacing in seed orchards and plantations for the future seed requirements in Queensland, Australia.

Light

Several experiments have tested the effects of light intensity, quality, and photoperiod on flowering in forest trees (Appendix 6). The effect of light intensity in open and closed stands was discussed in Chapter 3. Most of the older literature is related to similar field studies. However, more recently there have been experiments on the effects of light on flowering but, unfortunately, it is difficult to separate light and temperature effects.

One of the earliest experiments attempted to shorten the juvenile growth period in Betula by growing seedlings under continuous illumination, continuous long days, and normal daylengths in a greenhouse (Longman and Wareing 1959). After 10-12 months from seed, staminate flowers were initiated in plants grown under continuous light but no flowers were initiated in the other two groups. They concluded that maturity of trees resulted from the attainment of a certain size rather than the number of growth cycles. Since then, seedlings of Picea (Young and Hanover 1976) and Pinus contorta (Wheeler et al. 1982) have been grown under continuous light, and outplanted. Picea formed the first seed cones in the fourth growing season and Pinus contorta the first seed cones in the fifth growing season. In the latter case, cone enhancement carried over into the following year.

Photoperiod is generally considered not to have a direct effect on cone initiation in conifers (Ch. 3). However, some reports show a photoperiodic influence. Short days stimulated seed cone production in P. contorta (Longman 1961). It may be the length of the dark period that is critical in photoperiodic responses. Durzan and Campbell (1979), and Durzan et al. (1979), interrupted the dark period with red light and decreased seed cone initiation the following year on 10-year-old field grown Picea glauca. They tentatively concluded that this species is a short-day plant with respect to flowering but did not speculate on how this treatment could affect cone bud initiation the following year.

Based on a study of cone initiation in Thuja plicata under natural conditions and GA_3 induction, Owens and Pharis (1971) suggested that sex of cones might be affected by photoperiod because pollen cones were initiated during increasing daylengths and seed cones during decreasing daylengths. This has since been supported by an experiment using GA_3 to induce cones in Chamaecyparis nootkatensis. A higher proportion of pollen cones than seed cones were initiated under 16 hour daylengths than under 8 hour daylengths (Owens and Molder 1977f). However, temperature was not carefully controlled in this experiment. In Cryptomeria japonica, GA_3 treatment under long days (and high temperatures) promoted pollen cone initiation, whereas in Chamaecyparis obtusa long days and high light intensity promoted both pollen and seed cone initiation without GA_3 treatment (Nagao and Saskai 1981). In clonal grafts of Larix leptolepis seed cone buds were initiated only within a range of 12 to 16 hours of daylight but not below or above this range (Yokoyama and Asakawa 1973).

Photoperiod does affect shoot elongation in seedlings (Pollard and Portlock 1984), and it may be through the complex interaction between shoot elongation and reproductive bud differentiation that photoperiod has its effect. Slowing of vegetative growth appears to be a requirement for cone initiation in many conifers. Reproductive buds were induced on young Pinus taeda grown in a greenhouse under 20 hr daylengths by lowering the temperature and shortening the photoperiod. This slowed shoot elongation and caused a resting bud to form (Greenwood 1978a, 1981). The out-of-phase dormancy treatment may work on other hard pines in which seed cones are induced as growth slows in the late summer and fall. In Salix pentandra apical growth cessation in seedlings can be induced by short days, and apical growth cessation in mature trees is a prerequisite for floral initiation (Junttila 1980). In woody plants, only flowering in Ribes has been shown to be directly affected by photoperiod (Schwabe and Al-Doori 1973).

Other techniques

Floral induction may also occur as a result of branch pruning, grafting, bending, sheltering, and the use of cover crops (Appendix 7). Wareing (1953) observed in young Pinus sylvestris that branches on which the terminal bud had failed to develop due to insect attack the previous season bore numerous seed cones. A subsequent debudding experiment in May enhanced pollen cone initiation. Leader pruning (topping) of Pseudotsuga menziesii produced an increased crown area for cone production, reducing total cone production but not cone production per unit of height compared to controls (Copes 1973). The advantage of this method was in the ease of management of trees in seed orchards. Hedging for increased cone production and ease of collection has also been tried in conifers (Sweet and Krugman 1978). Pruning increased cone production in 15- to 18-year-old Pinus monticola but not in older trees; it appeared to increase the juvenile period in younger trees (Coffen and Bordelon 1981).

Grafting has resulted in precocious flowering on juvenile scions grafted to tops of some reproductive pines (Mirov 1951) but similar attempts have not been successful in other pines (Mergen 1962, Barnes and Bingham 1963). Increased cone production in scions from cone producing regions of the crown may result from slight incompatability (Ahlgren 1972) or poor graft unions causing partial gridling. Choice of rootstock also may be important in stimulating pollen or seed cone production in some conifers (Krusche and Melchior 1978).

Gravity and shoot orientation affect vegetative growth and flowering in some forest trees (Jackson and Sweet 1972). Generally, horizontal branches produce fewer vegetative and more reproductive buds than more upright branches. Branch bending has been used as a floral enhancement technique in fruit trees but the use in forest trees has been limited. Longman and Wareing (1958) found in L. leptolepis that the greater the branch angle down from the vertical the greater was the enhancement of reproductive buds. This treatment also brought young trees into heavy cone bearing (especially pollen cones) when they normally would produce few or no cones. Melchior (1961a, b) obtained similar results in the same species. Geotropic stimulation of cone induction has not been reported for other conifers. Tying down individual branches in Betula resulted in reduced shoot elongation but not increased flowering (Longman and Wareing 1958, Longman et al. 1965). One of the main benefits of this treatment may be to slow vegetative growth which in some species is necessary before floral initiation will occur (Wareing and Nasr 1958). The treatment may affect the distribution of growth substances but this aspect has not been fully investigated.

Increased floral initiation has occurred in individual young trees covered by polythene tents. This technique has been used successfully in B. verrucosa (Lepisto 1973) and Picea (Remrod 1972, Chalupka and Giertych 1977, Tompsett and Fletcher 1977) but increased cone initiation did not occur in Pinus sylvestris (Bergman and Kardell 1975). A positive response is thought to result primarily from increased day temperatures at the time of floral bud differentiation. More recently, placing potted scions and seedlings in polythene shelter houses has been successfully used as an adjunct treatment with drought and GA treatments (see section on GA treatments).

Growth regulator treatments

The review by Puritch (1972) discussed the application of various growth regulators to conifers to induce precocious flowering in juvenile trees or enhance flowering in sexually mature trees. Since that time there have been numerous reviews (Jackson and Sweet 1972, Pharis 1976, 1977, Pharis and Kuo 1977, Pharis and Ross 1976, Lee 1979, Dunberg and Oden 1983, Ross et al. 1983, Ross and Pharis 1984). About 100 research papers describe results utilizing a variety of species, growth regulators, concentrations of growth regulators, times and methods of application, plus several adjunct treatments. To discuss each paper would be extremely tedious, consequently a table (Appendix 8) lists most treatments, results, and references for attempted application of a growth regulator to forest trees. Reference to this table and the above mentioned reviews represent a minimum requirement to obtain an understanding of the potential breadth and complexity of these treatments. The text below will briefly review the types of growth regulators used. Since GAs are the only growth regulator treatments which have given promising results, at least in conifers, discussion will focus on them. GA treatments will be reviewed in terms of methods of application, time of application, and effects with adjunct treatments. Various theories on modes of action of GAs will be considered but only briefly, because research in this area is presently inconclusive.

Effects of various growth regulators

Puritch (1972) described the important role of auxins in the flowering of herbaceous plants but concluded that, in conifers, the significance of auxins in cone induction appears to be secondary through effects on juvenility and perhaps sexuality of cones. Hashizume (1969) studied endogenous auxins and GAs in nine conifers and concluded that seed cones are formed under higher auxin levels than pollen cones. Any direct role of auxins in promoting cone induction is uncertain; however, negative effects of auxin application on cone induction are common (Mann and Russell 1957, Hashizume 1959, Bonnet-Masimbert 1971, Brune 1973, McLemore 1975, Bleymuller 1976).

Although growth retardants may affect flowering in some orchard trees and woody ornamentals (Jackson and Sweet 1972), there are no reports of direct effects on floral initiation in forest trees. In conifers certain retardants, CCC (chlormequat) and ABA (abscisic acid), enhance cone induction but only when used in conjunction with $GA_{4/7}$ (Bleymuller 1976, Chalupka 1979, Ross et al. 1983). Similarly, cytokinins may enhance or modify GA responses in conifers but are not effective by themselves (Ross and Pharis 1984).

Gibberellins are thought to inhibit flowering in many woody angiosperms (Jackson and Sweet 1972), based on studies using GA_3. The application of more recently available non-polar GAs may give different results. Recent studies of apple (Pharis, pers. comm.) indicate that the less polar GAs may be effective in hardwood floral induction.

GA_3 was shown to induce cones in many species of the Cupressaceae and Taxodiaceae (Appendix 8). Both pollen and seed cone buds have been induced and, in many instances where these are initiated at different times, the sex has been manipulated by timing GA_3 treatments (Ross 1983b) or by varying the photoperiod during GA_3 treatment (Owens and Molder 1977f). Cone induction has been achieved in seedlings as young as three (Fraser 1970) or four months (Pharis and Morf 1967) and on individual branches of young trees (Ross 1983b). In the Cupressaceae and Taxodiaceae, there are no serious impediments to cost effective cone induction for increased seed production due to the following factors: GA_3 is relatively inexpensive; the treatments are easily applied as a foliar spray; the time required for treatments may be as short as three weeks; the trees may be manipulated regarding the sex of cones; and, resultant pollen and seed are of high quality (Sato 1963, Owens and Molder 1977f).

It was not until the mid-1970s that cone induction by GA treatment was achieved in the Pinaceae. This resulted from the use of the less polar $GA_{4/7}$ mixture (Hashizume 1973, Pharis et al. 1975, Ross 1975). Since that time, cone induction has been achieved in 16 species of Pinaceae representing five genera (*Larix*, *Picea*, *Pinus*, *Pseudotsuga*, and *Tsuga*). In most experiments, best results have been achieved when the $GA_{4/7}$ mixture was applied with some adjunct treatment (Appendix 8). Other non polar GAs (GA_5, GA_9) have been tried with some success but usually in combination with other GAs since pure preparations were not always available. However, the $GA_{4/7}$ mixture has been most effective (Tompsett 1977, Dunberg 1980, Pharis et al. 1980, Greenwood 1982). Progress has been slower in the Pinaceae than in the Cupressaceae because: less polar GAs are not as available and are more expensive than GA_3; treatments are not as easily applied (foliar sprays may not be effective); the timing of treatment is more critical; the length of treatment may be longer (commonly six weeks or more); $GA_{4/7}$ treatments are most effective when applied with adjunct treatments; and the sex of cones is not easily manipulated. Despite these problems, considerable progress has been made in the last five years in both field grown and containerized trees (Appendix 8).

Methods of GA application

The earliest and still most commonly used method for GA application is a water-based foliar spray containing a dilute carrier or wetting agent to facilitate penetration and variable concentrations of GA. In early studies Tween-20 at 0.1 per cent was commonly used as the wetting agent. It worked well for the Cupressaceae and

Taxodiaceae but not for the Pinaceae. Ross (1979) evaluated several foliar spray carriers for application of $GA_{4/7}$. More recently Aromox at 0.1 to 0.5 percent concentrations has been used successfully in the Cupressaceae and Pinaceae. A small amount of ethanol is frequently used to dissolve the GA before mixing with water. The concentration of GA used has been variable depending upon species and frequency and duration of applications. Original papers should be consulted for guidelines (Appendix 8).

Penetration appears to be through the cuticle of the leaf or young stem but the actual site of penetration has not been determined. Tritium (^3H) labelled GA_3 was applied as a foliar spray to *Thuja plicata* and foliage was studied using autoradiography (J.N. Owens, unpublished results). However, because GAs are water soluble, normal histological preparations remove the labelled GA. Freeze dried, or preferably freeze substituted tissue in which tissue preservation is better, will have to be used to prevent GA from being removed during histological preparations.

Foliar spraying is normally continued until the solution begins to drip from the foliage. This can be wasteful and costly, especially when applying expensive nonpolar GAs. Experiments using an aerosol powered chromatographic reagent atomizer which produces ultra fine mist (Ross 1983b) may provide foliar spray methods permitting the use of small amounts of GA with increased absorption into tissues.

Intravenous injection of aqueous solutions of GA have been used successfully, primarily in the Pinaceae (Appendix 8) and also in *T. plicata* (Coutts and Bowen 1973). This is most suitable for larger trees and field studies where foliar sprays are impractical, or in some species (eg. *Tsuga mertensiana*) in which foliar sprays have not been successful (J.N. Owens, unpublished results). In this method, solutions are fed from a bottle by a modified medical intravenous feeding unit into a hole drilled nearly through the main stem or branch (Ross 1978). Periodically, the bottle is refilled and a new hole drilled to prevent resin from plugging the hole and stopping the flow. This treatment translocates GAs primarily upwards from the point of injection. Other treatments employ single hypodermic injections under the bark (Pharis et al. 1969, Tompsett 1977, 1978b, Tompsett and Fletcher 1979) or injections into small holes bored into the stem. None of these methods provide a uniform, continuous flow of GA into the stem. Even the intravenous method used by Ross et al. (1985) required redrilling of holes about every 2 weeks and uptake was not uniform into a hole for more than a few days (Webber 1983).

Solutions containing GA (and other growth substances) have also been applied through small incisions in the bark of young stems, usually near the base of the terminal and distal lateral buds (Ross and Pharis 1976a, b, Ross et al. 1980, Kanekawa and Katsuta 1982, Katsuta 1981). Treatments must be repeated several times because only small amounts of GA can be applied at one time. Treatments can cause partial girdling which may enhance the treatment or even kill the shoot. GA applied in this manner is dissolved in ethanol and diluted with water containing no wetting agent. When Chalupka (1978) applied $GA_{4/7}$ in lanolin to 4-5 cm slits in the stem of *Pinus sylvestris* the proportion of branches bearing pollen cones increased. However, the time of his application, in May, suggests the effect may have been a result of partial girdling rather than a direct GA effect, since pollen cones do not differentiate until summer.

Topical application of GAs and other growth substances involves direct application of small quantities, often at fairly high concentrations, to the surface of young shoots (Pharis and Morf 1969, 1970, Ross 1975, 1976, Sweet 1979, Ross and Greenwood 1979, Greenwood 1981); to leaf traces after needles are removed from the branch (Puritch et al. 1979); or to the surface of buds (Pharis et al. 1975, Tompsett 1977, 1978b, Carson et al. 1977, Tompsett and Fletcher 1979, Sweet 1979, Hare 1979, 1984, Ross and Greenwood 1979, Wheeler et al. 1980, Greenwood 1981). These solutions commonly contain high concentrations of ethanol (70 to 80 per cent) which can be toxic to immature tissues. Therefore, although it is a useful experimental method for treating individual shoots, some damage and partial girdling may occur when application is made to young shoots, which could modify the GA effect.

Other methods, such as a soil drench of aqueous GA solutions (Pharis et al. 1969), have also been used.

There are no published reports of successful floral induction treatments using the above methods on hardwood forest trees.

Timing of GA application

The natural time of reproductive bud determination (Ch. 2) for a species under specific growing conditions is an important consideration often overlooked in early attempts at floral induction. Unsuccessful floral induction treatments commonly result from improper timing as well as improper method of application or choice of treatment. In many early experiments, treatments were applied over very long periods, whereas more recently, timing has been refined to coincide more precisely with known times of bud determination. This has permitted more exact, unambiguous experiments and less costly floral induction (Appendix 8).

In most forest trees we do not know when potentially reproductive apices begin biochemical differentiation which leads to morphological differentiation. In Pseudotsuga menziesii histochemical changes begin five weeks before easily recognized morphological differentiation (Owens 1969). This time is long compared to herbaceous angiosperms, where biochemical changes occur only hours or days before morphological differentiation (Bernier 1971). In conifers it is generally agreed that as yet unknown biochemical changes leading to bud initiation precede morphological changes (Dunberg 1979). Therefore, at the present time, we can only estimate the optimal time for GA application based on the onset of morphological differentiation. If more precise techniques, such as immunocytochemistry, can be developed to identify the earliest biochemical changes, then more precise timing of GA application will be possible. However, just how contracted the period of application can be for floral induction depends upon the rate of GA uptake, metabolism of GAs in the tissues, and mode of action of GA in the flowering process. Presently, little is known about these aspects (Ross and Pharis 1985).

Possible modes of action of GA in flowering

For many years it has been recognized that GAs induce flowering in long day plants (Lang 1957) and in plants requiring low temperatures (Chailakhyan 1961). They may also be essential in floral initiation in short day plants (Hodson and Hamner 1970). Chailakhyan (1961, 1968) theorized that GA is a major component of florigen, with GA controlling growth and development and anthesins controlling floral initiation, both required for flowering. Lang (1956) proposed that GAs initiate flowering by stimulating stem growth or factors associated with growth.

Pharis and Kuo (1977) reviewed the literature on endogenous GAs of conifers, processes correlated with changes in GA-like substances, and processes affected by exogenous application of GA. More recently Dunberg and Oden (1983) also have reviewed the metabolism of GAs with respect to conifers. Floral induction is only one of the several growth and developmental processes affected by GA but the mechanism by which the responses are evoked is still unsettled. There are other recent reviews dealing with the biochemistry of GAs with respect to flowering in general (Zeevaart 1983). The biochemistry of the more than 60 known GAs and their mechanism of action in reproduction are not fully understood and a detailed discussion is beyond the scope of this review. However, a brief discussion of the rationale behind the successful use of different GAs and hypotheses regarding their action is warranted.

The early success in floral induction using the more polar GAs (e.g. with more than one hydroxyl) in the Cupressaceae and Taxodiaceae and later success using less polar GAs (e.g. with only one hydroxyl) in the Pinaceae, probably relate to the levels of native GAs within the tree, the metabolism of GAs including their complex and often rapid interconversions, and their mode of action. Few studies have analysed changes in endogenous GAs during the natural time of floral bud differentiation in conifers (Dunberg 1976, Lorenzi et al. 1976, McMullan 1980) or woody angiosperms (Leshem and Ophir 1977). However, extensive research is underway on GA metabolism with emphasis on the flowering processes in conifers and certain woody angiosperms (Pharis, pers. comm.).

Some alternative hypotheses have come from recent research relating exogenous application of GAs, endogenous levels of GAs, and floral induction. One hypothesis is that vigorously growing conifers utilize endogenous GAs preferentially for vegetative growth, and it is only when environmental or other factors restrict this growth that GAs are available for floral initiation (Pharis 1976). Exogenously applied GAs of the proper form, for that species, may increase the endogenous levels of certain GAs, so that flowering will occur in otherwise vegetative trees.

Another hypothesis, derived from Sachs (1977), emphasizes the availability of assimilates and essential nutrients in the apical meristems. Sachs postulated that flowering may be evoked by diversion of nutrients to the apical meristem. It has been suggested that exogenous application of GAs enhances the flow of assimilates to the buds where floral differentiation occurs (Ross et al. 1985). In a recent study GA$_{4/7}$ enhanced flowering in *Pinus radiata* and caused a significant reallocation of dry matter and ^{14}C-photosynthate to potential seed cone primordia within the long shoot bud (Ross et al. 1984).

A third hypothesis is based on relative growth rates and distribution of meristematic activity determining whether a shoot apex will become vegetative or reproductive (Romberger and Gregory 1974, Tompsett 1978a). Tompsett and Fletcher (1979) suggested that application of growth regulators promotes cone induction by enhancing the early growth rate of potentially reproductive buds. Tompsett (1978a) formulated his hypothesis by comparing bud vigor in different regions of the crown rather than by comparing potentially vegetative and potentially reproductive apices on comparable branches. Recent results with *Pseudotsuga menziesii* (Owens et al. 1985) show that trees on which apical mitotic activity and growth were inhibited by rootpruning and rootpruning plus GA$_{4/7}$ treatments produced greater numbers of cones than control and GA$_{4/7}$ treated trees (Ross et al. 1985) on which buds were not inhibited.

A fourth hypothesis is that, in cone induction treatments, exogenous growth regulators (GAs at least) are applied and taken up in amounts far exceeding that required for reproductive bud differentiation, and induction results not from a direct morphogenic effect but more from a stress effect (McMullan 1980, Dunberg and Oden 1983). This is based on estimates by McMullan (1980) that the level of GA$_{4/7}$ taken up by shoots is 5000 times higher than in untreated shoots. She points out that even with dilution by the expanding shoot and metabolism of GAs, the concentration in differentiating buds would likely be considerably higher than normal. However, the amount of GA$_{4/7}$ actually in differentiating buds has not yet been determined. Until this is done we do not know if these really are superphysiological doses of GA (Reeve and Grozier 1975). Research indicates that only a small proportion of applied GAs are absorbed (Ross and Pharis 1982) and the developing buds have access to only a fraction of that. Results using tritium-labelled GA$_4$ in *Pseudotsuga menziesii* indicate that over 95 per cent of the GA$_4$ moved to adjacent stem and needle tissue (R.P. Pharis, J.G. Webber, S.D. Ross and J.N. Owens, unpublished results) and that [^{3}H] GA$_4$ is rapidly mobilized and converted to inactive forms (Wample et al. 1975).

Tests of these hypotheses are far from conclusive. However, research is underway in various laboratories which may test them in a definitive way. Results should provide useful information about the roles of GAs, and other growth regulators, on floral initiation.

GA with adjunct treatments

Although GA application alone may induce flowering in some forest trees, the most successful cone induction has been achieved using GAs with one or more of the previously discussed adjunct environmental or cultural treatments (see Appendices 1-8). The response to combined treatments is commonly synergistic rather than additive. The number of potential combinations is almost limitless and results to date have been extremely variable (see Appendix 8). Adding only one adjunct treatment more than doubles the complexity of the treatment since it introduces variables such as severity of the treatment and timing, in addition to the synergistic effects. If too many variables are introduced it becomes impossible to sort out all of the interactions.

All experiments will not be discussed; rather, Appendix 8 lists for each species the type of GA treatment, the adjunct treatment(s), the results, and references. Reference to this list and the literature cited for each species is essential before floral induction is attempted for a species. The purpose of this section is to provide a brief historical perspective to combined treatments and point out combined treatments which have been most effective.

In the Cupressaceae and Taxodiaceae, GA_3 foliar sprays usually result in abundant reproductive buds in seedlings or young trees. Consequently, adjunct treatments are not needed unless manipulation of the proportion of pollen and seed cone buds is desired. In Thuja plicata (Pharis and Morf 1970) and Chamaecyparis nootkatensis (Owens and Molder 1977f) GA_3 application under short days enhanced seed cone bud initiation, whereas application under long days enhanced pollen cone bud initiation (Ch. 2, Light). C. obtusa sprayed with GA_3 under varying light qualities but constant light intensity showed a light quality effect on the induction and sex of cones (Nagao 1983a). Ethrel has been used with GA_3 and has generally enhanced cone induction in Cupressus arizonica and Chamaecyparis lawsoniana (Bonnet-Masimbert 1971) and C. obtusa and Cryptomeria japonica (Hashizume 1975).

In the Pinaceae, $GA_{4/7}$ application alone may induce or enhance reproductive buds, whereas combined treatments commonly give a marked synergistic effect. Many of the earliest treatments combine $GA_{4/7}$ with some form of girdling (Ross 1975, 1978, Pharis et al. 1975, Ross and Pharis 1976a, b, Puritch et al. 1979). Girdling generally increased the $GA_{4/7}$ effect but in some studies there was a decrease in seed set with girdling (Ross 1975, Ross et al. 1980). Because of this, and the fact that girdling may permanently damage costly seed orchard trees, this combined treatment should perhaps be restricted.

Rootpruning has been successfully used alone (Ch. 3) and as an adjunct treatment with $GA_{4/7}$ on larger trees (Greenwood 1977, Ho 1982, Ross et al. 1985). The severity of rootpruning has varied and it has not yet been determined if the rootpruning effect is one primarily of reducing water uptake, resulting in moisture stress, or if there is also a root factor involved in flowering. There may be considerable lag-time in the rootpruning effect. Rootpruning done in late March increased moisture stress in field grown P. menziesii from early April until early June (Ross et al. 1985). In that study, there was a synergistic effect of $GA_{4/7}$ and rootpruning on cone induction (Ross et al. 1985). Rootpruning slowed mitotic activity and development of vegetative terminal buds (Owens et al. 1985) and potentially reproductive buds (Owens et al. 1986) and delayed the time of bud differentiation. This treatment also significantly increased flowering. Rootpruning plus $GA_{4/7}$ delayed bud differentiation to the same extent or slightly more and increased flowering more than either treatment given on its own. The effects of these treatments on endogenous GAs is still under investigation (R.P. Pharis, pers. comm.). With the exception of the above P. menziesii study (Ross et al. 1985, Webber et al. 1985, Owens et al. 1985), comprehensive studies of treatment interactions and effects on development and biochemistry of shoots and buds have not been done but are essential to an understanding of the comparative roles of combined treatments.

The use of containerized trees in GA cone induction experiments allows more careful control of moisture stress than rootpruning. Carefully controlled moisture stress in field grown trees is not possible under most conditions. Moisture stress (Ch. 3) can be reasonably well controlled by careful watering and measurement of shoot water potential, using the pressure chamber technique (Ritchie and Hinkley 1975). Unmonitored moisture stress can give poor results because, although watering may be restricted, soil moisture, humidity, and temperature may reduce anticipated moisture stress giving poor synergistic effects with $GA_{4/7}$. At the other extreme, it is easy to overstress and kill many species.

Combined effects of moisture stress and $GA_{4/7}$ were first used to induce cones in containerized Pinus taeda (Greenwood 1978b) and Pseudotsuga menziesii (Ross 1978). Since then the technique has been used successfully in containerized

Picea sitchensis (Philipson 1983), *P. glauca* (S.D. Ross, pers. comm.) and *Tsuga heterophylla* (Ross et al. 1981, Pollard and Portlock 1981a, b, 1983, Brix and Portlock 1982, Rottink 1982). The advantage of using containerized trees in moisture stress and GA$_{4/7}$ treatments is that treatments can be carefully controlled. Also, once cone-buds have formed, subsequent cone development can be optimized by careful maintenance of the containerized trees. The use of containerized trees, especially when grown in shelterhouses or some other modified environment, often alters the phenology. Treatment times should be correlated with shoot elongation or bud development rather than calendar date, to ensure that treatments begin before floral differentiation begins (Ch. 2). Because GA$_{4/7}$/moisture stress experiments should be carried out under some type of cover in order to control moisture, temperature and light may become confounding factors.

Increased temperature accompanying GA$_{4/7}$ treatments has been used to induce cones in several conifers (Appendix 8). However, temperature has usually not been carefully controlled and has been increased simply by placing trees in greenhouses or shelterhouses or by placing polythene tents over trees grown in the field. In one carefully controlled experiment, 3-year-old *T. heterophylla* seedlings were grown in growth chambers with daytime temperatures of 20, 25, 30, and 35°C and treated with GA$_{4/7}$ and moisture stress (Pollard and Portlock 1981a). There was an increase in both pollen and seed cones with GA$_{4/7}$ and increasing temperature up to and including 30°C. Moisture stress approximately halved the effect at 25°C but doubled the effect at 30°C. In *Cryptomeria japonica* sprayed with GA$_3$, more pollen cones were initiated under 30°C days and 25°C nights with day temperatures having greater influence, whereas more seed cones were initiated under lower temperatures (20°C day and 15°C night) (Nagao 1983b). Tompsett and Fletcher (1979) and Philipson (1983) increased the efficacy of GA$_{4/7}$ and GA$_9$ when these were applied to *P. sitchensis* grafts in a polyethylene house. The use of polyethylene houses or covers to increase temperature has also increased the efficacy of GA$_{4/7}$ in *P. abies* (Luukkanen 1979) and *Pseudotsuga menziesii* (Bonnet-Masimbert 1982). The use of increased temperature to increase the efficacy of various GA (and other) treatments is promising, especially for small containerized trees. In this way many cones can be produced in small areas in relatively few years.

There have been few experiments in the Pinaceae using the combined effects of GA$_{4/7}$ and photoperiod or light intensity. Longman (1982) found that GA$_{4/7}$ application under short days enhanced female, but not male, flowering in *Pinus contorta*. Pollard and Portlock (1984) applied GA$_{4/7}$ to *T. heterophylla* and increased male flowering under long days and female flowering under short days. Greenwood (1981) used 20 hr photoperiods followed by natural short days to induce an out-of-phase dormant period in *P. taeda*. This treatment alone promoted both male and female flowering, whereas GA$_{4/7}$ plus moisture stress promoted female flowering only. It appears, at least in hard pines, that the promotion of a resting bud induced by short photoperiods is an essential first step in cone induction. GA$_{4/7}$ (and moisture stress) affected bud size and the course of axillary bud differentiation. Out-of-phase dormancy treatments may only be effective in some species of hard pines where considerable differences occur in the natural photoperiods when pollen and seed cone buds differentiate.

Extended photoperiods have been used to accelerate growth and reduce the juvenile period of growth (Young and Hanover 1976, Wheeler et al. 1982). Cecich (1981) demonstrated that *P. banksiana* seedlings cultured under accelerated growth conditions for several months, and then treated with GA$_{4/7}$ (and NAA), had a fourfold increase in seed cones over seedlings receiving only accelerated growth treatments. The use of accelerated growth plus GA$_{4/7}$ may be a useful tool in breeding programs of species having a long juvenile growth phase.

GA$_{4/7}$ has been used in combination with other plant growth substances (Appendix 8). In general, auxins and cytokinins which are ineffective on their own may enhance or modify the response of GAs (Pharis and Kuo 1977, Ross et al. 1983). There is some evidence that combined growth substances may influence the proportion of male and female flowering (Ross 1976, Hall 1977, Tompsett 1977, 1978b, Puritch et al. 1979, Bonnet-Masimbert 1982). Presently their limited benefits may not warrant their inclusion in most cone induction treatments. We need to know much more about growth regulator combinations for floral induction.

Also, GAs have been used, together with various fertilizer treatments, often in combination with cultural treatments (Appendix 8). Calcium nitrate ($Ca[NO_3]_2$) has been used in several studies (Ross et al. 1981, Pollard and Portlock 1981a, b, 1984); it generally enhances the GA$_{4/7}$ effect. In Pseudotsuga menziesii, Ross (1978) found that nitrate was more effective than ammonium as a source of nitrogen in promoting pollen cone induction in response to GA$_{4/7}$ (and moisture stress). Fertilizer application in conjunction with GA$_{4/7}$ may have some effect on GA efficacy and the sex of cones initiated. Fertilizer is too easily added to several other treatments with often small and inconclusive results. The goal of many of the early experiments was to induce cones by any means rather than a careful evaluation of a few variables. This makes evaluation or replication of these experiments difficult.

Summary, and recommendations for future research

There are many ways of enhancing flowering both in reproductively mature trees and in juvenile or otherwise non-flowering trees. It has generally been easier to accomplish the former than the latter. Many rather simple cultural treatments used alone or in combination will enhance flowering. Most cultural treatments are not long lasting and must be repeated for each new crop. Some carry an element of risk and may damage or kill treated branches or trees. Many cultural treatments are only successful in enhancing the natural flowering cycle of a tree and have little or no effect in off years. This, combined with improper methods and timing of treatments, has led to many contradictory results. Cultural treatments must be applied before the natural time of floral initiation, although the specific times of application have not yet been determined for most species. Lag time for the treatment to take effect must also be considered. Superimposed upon these variables are variations in development, natural cycles, and physiology of different species. It is not surprising that even the simplest cultural treatment does not always produce the desired results.

One of the most recent and promising floral enhancing and inducing treatments in conifers is the use of GAs. GA$_3$ has long been successfully used in the Cupressaceae and Taxodiaceae but more important are the recent successes using non polar GA$_{4/7}$ mixtures in the Pinaceae. As with cultural treatments, there is considerable variation in results because of differences between species, timing, methods of application, and form of GA used. The mode of action of GAs in floral induction is still unsettled and their biochemistry and metabolism are complex. Experiments in this area require exacting laboratory techniques. Other growth regulators are not effective in promoting flowering in conifers but may alter GA effects. So far GAs appear to be ineffective in floral induction of hardwoods, whereas some growth retardants are effective in some fruit orchard species. Development of optimal combinations of cultural and GA treatments should eventually allow early or enhanced flowering of most conifers. This will provide the tree geneticist/breeder and seed orchardist with valuable techniques for obtaining precocious flowering and abundant genetically improved seed for reforestation.

Combinations of cultural and GA applications often produce dramatic results even in juvenile and poor flowering individuals. The number of possible combinations of treatments is almost unlimited and variations in results from combined treatments reflect this. Combined GA and cultural treatments often give synergistic results and many adjunct treatments appear to increase the efficacy of the GA.

Experiments on the precise timing of environmental, cultural, and growth regulator treatments should be preceded by studies of the time of floral initiation. The time and duration of treatments for optimal flowering can be far more refined than it now is for most species. Some exceptions are in the Cupressaceae and perhaps Tsuga heterophylla of the Pinaceae. Timing must be correlated to an easily monitored and predictable morphological feature such as lateral shoot elongation in the Pinaceae. This needs to be carefully done in all species and may initially require anatomical study of bud and shoot development during treatments.

Although research on floral enhancement in hardwoods is almost non existent and should be established on poor or late flowering species, research on conifers is progressing rapidly. More emphasis should be given to research on floral induction of containerized clonal and seedling material as opposed to poorly controlled field experiments. The use of containerized trees has been very successful for several conifers and this technique should be applied to hardwood species. Abundant flowering can be produced on small trees in a limited space although the cost effectiveness of this technique should be determined. Early accelerated growth of containerized juvenile stock followed by floral induction treatments is also promising. Containerized stock is ideal for floral induction experiments in carefully controlled environments using one or a combination of treatments. With this approach, the many ambiguities present in the literature may be clarified and costly repetition of past errors avoided. Research on hardwoods should perhaps begin on containerized stock rather than starting on field grown trees.

Treatment(s) required for optimal flowering of each commercially important species should be determined. Optimal flowering refers to sexuality as well as number of flowers and the capacity of the tree to carry cones or fruits through to maturity without excessive abortion. In the Pinaceae there is a need to develop methods for pollen cone induction in juvenile trees.

GA$_3$ treatments in the Cupressaceae and Taxodiaceae are very effective, but more research is needed to reduce the duration of treatment and to determine precise timing to optimize cone induction and manipulate the sex of cones. Experiments using GA$_3$ under different photoperiods in controlled environemnts should provide useful information on the control of cone sexuality.

In the Pinaceae cone induction by GA$_{4/7}$ combined with cultural treatments is rapidly becoming effective. However, timing of application is more critical than in the Cupressaceae because cone bud differentiation appears to be related to the fixed stages of bud development in the Pinaceae. More research under carefully controlled conditions using GA$_{4/7}$ with limited cultural treatments is needed to refine the technique so it will be repeatable and cost effective. Developmental studies accompanying experiments on containerized stock are required in order to determine the optimal times and methods of application and the causes of good or poor cone induction.

Research should continue on the refinement of fertilizer applications to induce and enhance flowering in conventional seed orchards. More effective timing of treatments and forms of application may be developed. Rootpruning is effective in young seed orchards but results are still quite variable. Methods vary, as do conditions within individual seed orchards, time of treatment, and species response. Experiments must continue but should be tailored to specific local problems, species, and conditions. For example, rootpruning may be essential on wet sites but detrimental on dry, stressful sites.

Some basic research should be done to determine the time of the earliest biochemical changes associated with floral initiation. Immunocytochemical techniques could be adapted to studies of floral initiation in forest trees as they have been in herbaceous angiosperms. This could pinpoint exact times of floral initiation for timing of floral induction treatments.

The role of GAs in flowering is not known, although several theories have been proposed. Basic research must continue on the native GAs (and other growth regulators) within trees as they pass from the juvenile to the reproductive phase and during the annual growth cycle. Also, the fate of exogeneously applied GAs must be determined because there are many forms of GA and their metabolism is poorly understood. These could be determined using radioactively labelled GAs and autoradiography of freeze substituted tissues. The effect of various floral inducing cultural treatments on endogenous growth regulators must be determined. The relationship between flowering and changes in carbohydrates and certain amino acids should be investigated. Although the primary goal is to induce flowering in forest trees, it is important to understand how the inductive treatments affect the flowering process.

Most floral induction researchers are confident that seed resulting from induced cones is comparable in quality to that from non-induced trees. This assumption results from the fact that most induction treatments are of short duration and all precede seed maturation by one or more years. However, excessive flowering can result in ovule, seed, flower, and cone abortion and perhaps seed of poor quality due to competition for resources. This may be especially true of small trees but may be most easily overcome in containerized trees. Trees have limits to their ability to support seed production. Therefore, tests should continually be made of the seed produced to establish convincing data to support the contention that high quality seeds result from floral induction treatments.

CHAPTER 5

POLLEN AND POLLINATION

Introduction

In all north temperate conifers, microsporangia (pollen sacs) are initiated within the pollen cone buds before winter dormancy (Ch. 2). However, the stage of microsporangial development reached before dormancy varies among species. Most temperate hardwoods appear to initiate flowers before winter dormancy, but the stage of floral development reached is inadequately described. Development following dormancy is rapid. In most forest species, meiosis, microsporogenesis, pollen development, and pollination occur within a few weeks or months. Pollen development, pollen morphology, and pollination mechanisms are different in conifers and in hardwoods, and species variations occur within each group. The time and duration of pollination vary with the species and with latitude, elevation, site, and weather. Many of the stages of pollen development and pollination may be managed to increase seed production.

Meiosis and pollen development

Conifer pollen cones initiate many microsporophylls, each bearing two (Pinaceae) or more (Cupressaceae and Taxodiaceae) microsporangia on their abaxial (away from the cone axis) surface. In angiosperms each stamen forms two anthers, each consisting of two fused microsporangia (Foster and Gifford 1974). Within each microsporangium, many sporogenous cells form, each of which undergoes meioses to form a tetrad of microspores. Each microspore develops into a pollen grain (Owens 1982).

Conifer pollen cones overwinter at different stages of development (Fig. 5.1). Overwintering may begin when pollen cones are at the sporogenous stage as in Pinus (Kupila-Ahvenniemi et al. 1978, Owens and Molder 1977c, Owens et al. 1981b), or at the pre-meiotic pollen mother cell (PMC) stage as in Picea and Abies (Sarvas 1974, Moir and Fox 1975, Owens and Molder 1977d, 1979a, 1980a, Singh and Owens 1981a, b, Harrison and Owens 1983). In these genera, meiosis and pollen development occur after winter dormancy. Ultrastructural studies of overwintering sporogenous cells of Pinus (Kupila-Ahvenniemi et al. 1978, Cecich 1984) and PMCs of Pseudotsuga (Singh et al. 1983) have shown that a true "dormant" period does not occur; rather, nuclear and cytoplasmic changes occur throughout the winter. This period is more correctly called a period of reduced activity rather than dormancy. Similar studies have not been made for Abies or Picea.

In Larix, Pseudotsuga, Thuja, and Tsuga, meiosis begins in the fall, then becomes arrested when PMCs reach either the pachytene or diffuse diplotene stages of meiosis (Eriksson 1968a, Eriksson et al. 1970a, b, Owens and Molder 1971a, b, Hall 1982). After dormancy meiosis is rapidly completed, followed by pollen development.

In Chamaecyparis and Juniperus, meiosis and pollen development occur before winter dormancy (Owens and Molder 1974b). Overwintering pollen cones contain mature, dry pollen, and no structural changes have been observed with the light microscope during winter.

Winter temperatures may affect pollen cone buds and pollen quality (Eriksson et al. 1970a). A higher incidence of pollen abnormalities occurred in Larix growing in Sweden, when PMCs developed beyond the diffuse stage before winter dormancy (Ekberg et al. 1967, Eriksson 1968a, b). Consequently, trees moved to seed orchards in colder areas may have a higher incidence of pollen inviability or abnormalities.

Luomajoki (1982) reported the dates for the onset of meiosis following winter dormancy in seven conifers and four hardwoods. He concluded that there was no abrupt thermal threshold necessary to start meiosis in the spring and that Q_{10} factors (differences in developmental rate within a range of 10°C) are of little value as delineators of meiotic development. Only Sarvas (1972) has gathered sufficient data to demonstrate a regression using temperature which is valid for meiosis.

Several studies have followed the normal sequence of meiosis in conifer PMCs using the light microscope (Zenke 1953, Mergen and Lester 1961, Mergen et al. 1963, Chandler and Mavrodineanu 1965, Livingston 1971a, Moir and Fox 1975, Ho and Owens 1974a, b, Seido 1979, Luomajoki 1982), and premeiotic and early miotic stages using the electron microscope (Willemse 1971a, b, Dickinsen and Bell 1976, Kupila-Ahvenniemi

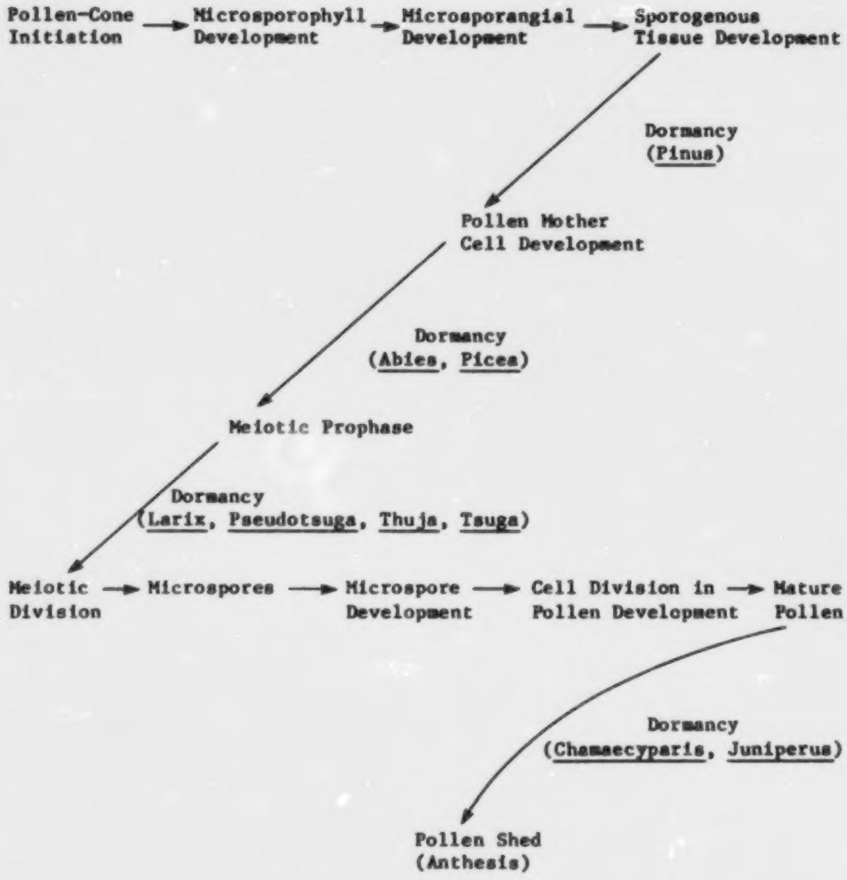

Pollen-Cone Initiation
Microsporophyll Development
Microsporangial Development
Sporogenous Tissue Development
Dormancy (Pinus)
Pollen Mother Cell Development
Dormancy (Abies, Picea)
Meiotic Prophase
Dormancy (Larix, Pseudotsuga, Thuja, Tsuga)
Meiotic Division
Microspores
Microspore Development
Cell Division in Pollen Development
Mature Pollen
Dormancy (Chamaecyparis, Juniperus)
Pollen Shed (Anthesis)

Figure 5.1 Stages of pollen and pollen cone development times when dormancy may occur in different species (from Owens 1982).

et al. 1978, Singh et al. 1983, Cecich 1984). Vasil (1978) reviewed the limited literature available on the ultrastructure of premeiosis, meiosis, and pollen development in gymnosperms. Little work has been done on meiotic stages of hardwoods (Luomajoki 1977, 1982).

The meiotic process is complex, making it susceptible to environmentally caused irregularities which may result in reduced pollen viability or vigor. Several experiments have shown that low (Christiansen 1960, Eriksson 1970, Jonsson 1974, Luomajoki 1977, Andersson 1980) and high (Sarvas 1972) temperatures cause meiotic irregularities in conifers. The physiological condition of the tree may also affect pollen abortion. Chandler and Mavrodineanu (1965) found increased pollen abortions due to meiotic irregularities in trees growing on very dry sites. Many irregularities are believed to be reversible (Jonsson 1974) and cause no permanent damage, whereas others may result in abnormal pollen and pollen of low vigor or viability. Luomajoki (1977) believes low temperatures may lead to irreversible damage causing permanent meiotic irregularities. Relatively high doses of γ-irradiation have also induced a variety of chromosomal aberrations and pollen abnormalities in Larix (Erriksson et al. 1966). The frequency of meiotic irregularities is generally low but under some conditions may exceed 50 per cent of the PMCs (Jonsson

1974). An experiment on the effect of low temperatures on pollen development of four *Larix* species resulted in 92 and 84 per cent pollen sterility in *L. decidua* and *L. siberica*, respectively (Ekberg and Eriksson 1967).

Following meiosis the tetrads of haploid microspores remain within the PMC wall for a brief time. They soon swell, burst out of the PMC wall, and become suspended in fluid within the microsporangium where subsequent pollen development occurs (Singh 1978).

Two patterns of cell division occur during pollen development in north temperate conifers (Figs. 5.2, 5.3) (Sterling 1963, Singh 1978). In the Pinaceae the microspore divides unequally, forming a small lens-shaped prothallial cell and a large embryonal cell. The embryonal cell then divides unequally forming a second small prothallial cell and a large antheridial initial. Prothallial cells have no known function. The antheridial initial divides to form a large tube cell and a small generative cell. Pollen may be shed at this four celled stage or at the five celled stage, after the generative cell divides equally to form the stalk and body cells (Fig. 5.2). The body cell forms the two male gametes after pollination (Ch. 6). In the Pinacae, pollen is generally large, sacci (wings) are present in some genera (Figs. 5.4, 5.5, 5.6) but not in others (Figs. 5.7, 5.8), and storage products are in the form of starch (Sterling 1963, Owens 1982, Owens and Molder 1971b, 1975c, 1977c, d, 1979a, b, 1980a, Owens et al. 1981b, Singh 1978, Singh and Owens 1981a, b). The Podocarpaceae and Araucariaceae, mostly from the southern hemisphere, have similar development except that the prothallial cells continue to divide forming non- functional prothallial tissue (Singh 1978).

In the Cupressaceae, Taxodiaceae, and Taxaceae, pollen has no prothallial cells. Each microspore divides forming a tube cell and generative cell (Fig. 5.3) and pollen is shed at the one or two celled stage. The generative cell forms two male gametes after pollination. In these families,

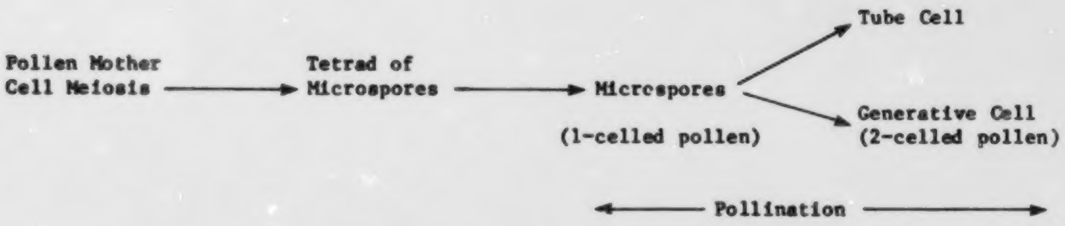


Figure 5.2 Pollen development in *Chamaecyparis*, *Juniperus*, *Taxus*, and *Thuja* (from Owens 1982).

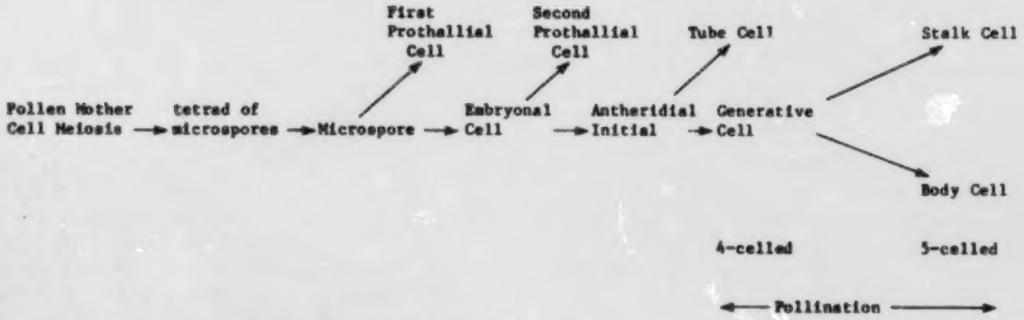
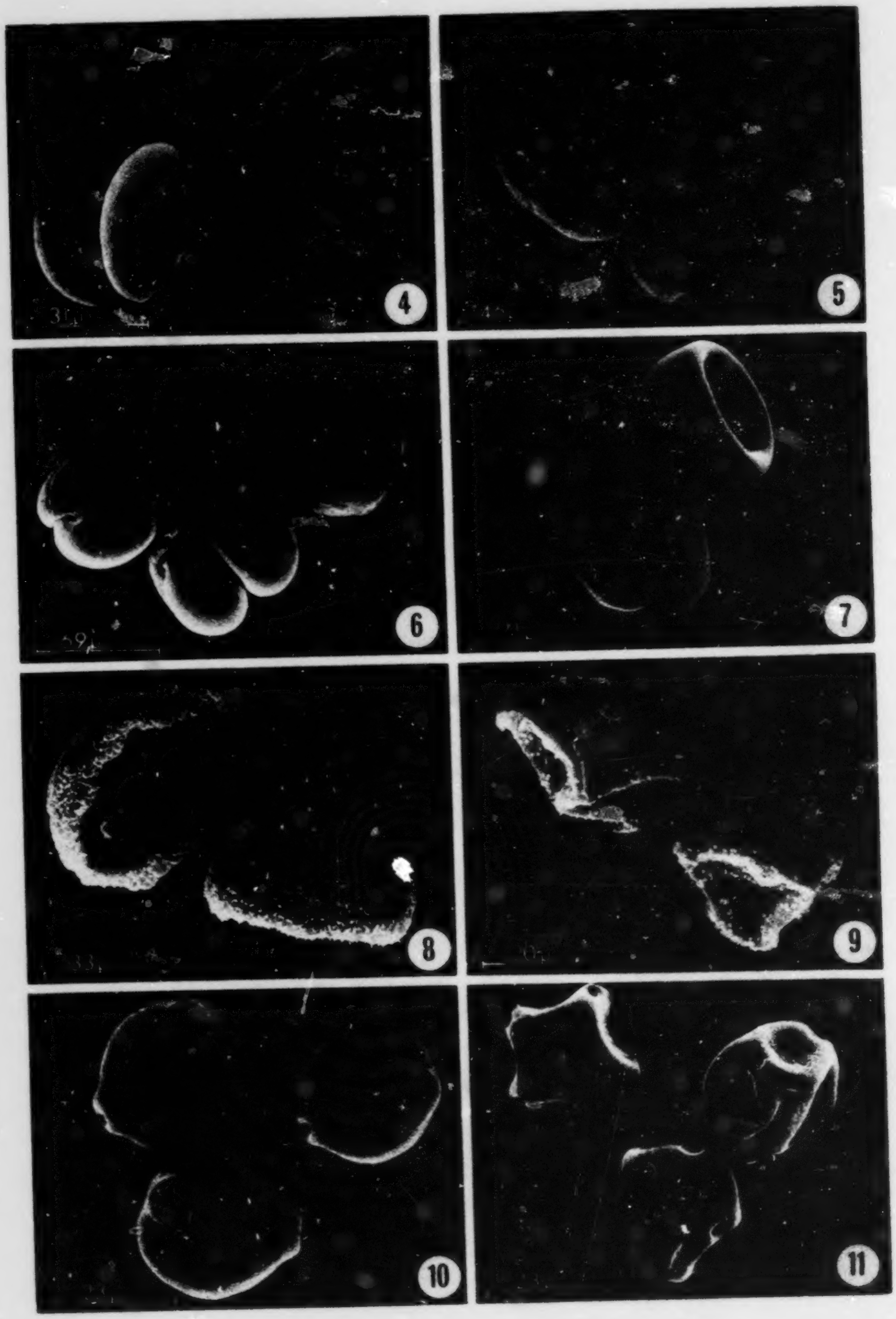


Figure 5.3 Pollen development in *Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, and *Tsuga* (from Owens 1982).



Figures 5.4 - 5.11 Scanning electron micrographs of pollen.
Fig. 5.4. *Pinus banksiana*. Fig. 5.5. *Picea glauca*. Fig. 5.6. *Abies lasiocarpa*. Fig. 5.7. *Pseudotsuga menziesii*. Fig. 5.8. *Tsuga heterophylla*. Fig. 5.9. *Thuja plicata*. Fig. 5.10. *Betula*. Fig. 5.11. *Alnus*.

pollen is small, lacks sacci, is sculptured with orbicules (Fig. 5.9), and the storage products are oil droplets (Sterling 1963, Li 1975, Owens 1982, Owens and Molder 1974b, Owens et al. 1980, Singh 1978).

In hardwoods pollen development is similar to the latter type and pollen is shed at the two or three celled stage. Most angiosperms shed pollen at the two celled (binucleate) stage, containing a vegetative and a generative cell. The latter cell forms two male gametes after germination. The three celled (trinucleate) pollen contains a vegetative cell and two male gametes. Trinucleate pollen has all of the precursors for pollen tube growth, and germination commonly occurs within minutes (Heslop-Harrison and Heslop-Harrison 1982). Germination and pollen tube growth is generally slower in binucleate pollen (Cresti et al. 1977). There is considerable information about ultrastructural and physiological changes during germination and pollen tube growth in herbaceous plants (see Cresti et al. 1977, Heslop-Harrison and Heslop-Harrison 1982) which are beyond the scope of this review, but there is little information on hardwood forest trees. Only pollen development of Quercus (Conrad 1900, Larson 1965) and Populus (Nagaraj 1952) have been studied in detail and both are binucleate. Quercus pollen is described by Olsson (1975). Pollen morphology is extensively described in palynology texts. Hardwood pollen is generally small, non-saccate, has conspicuous pores, and is commonly ornately sculptured on the surface (Figs. 5.10, 5.11) (Foster and Gifford 1974).

Most stages of meiosis and pollen development occur after winter dormancy. The time sequence varies among species but can be separated into five stages of development: pre-meiotic division, meiotic division, microspore development, cell division, and anthesis. The time spent at each stage varies among species and with the weather. The time from the end of pollen cone bud dormancy to anthesis may be as little as one week in Chamaecyparis nootkatensis, in which mature pollen forms before winter dormancy (Owens and Molder 1974b), to 12 weeks in Tsuga mertensiana (Owens and Molder 1975c). In Thuja plicata pollen cones require a sequence of short days and cold followed by long days before anthesis occurs (Pharis et al. 1969, Simak et al. 1974). The phenology of pollen development for several conifer species is given in Figure 5.12.

We do not know the effects of temperature on the rate of development of the different stages. However, increased temperatures will shorten the total time to anthesis (Sarvas 1962, 1965, Winton 1964, Boyer and Woods 1973), and forcing pollen for early pollen extraction is possible. In Pinus palustris bagging branches increased the degree-hours of heat accumulated and advanced flowering an average of 8.6 days ahead of unbagged controls (Boyer and Woods 1973). The longer the period of postdormancy development (Fig. 5.12), the more potential exists for pollen forcing.

Studies of pollen development and pollen quality should accompany pollen forcing trials. High temperatures, that cause meiotic irregularities (Sarvas 1972), may also cause irregularities in pollen development. Abnormal pollen has been observed in many species (Hutchinson 1915, Mehra and Dogra 1965, Diaz Luna 1977, Ho and Owens 1974a, c, Singh and Owens 1982) as has variation in pollen viability (Ekberg and Eriksson 1967). These irregularities may result from various causes, including temperature. Luomajoki (1977) cautions that very accurate control and measurement of temperature are needed in pollen forcing experiments. Sarvas (1972) describes forcing cabinets that provide for precise measurement. Forcing too rapidly may reduce food reserves and the vigor of pollen by shortening the period during which reserves are accumulated. There are no published reports of these types of studies in forest trees but one is underway for Tsuga heterophylla (A. Colangeli, pers. comm.). Generally, the long period of post-meiotic pollen development makes it possible to control the rate of development in many species but the extent to which pollen can be manipulated before development, vigor, or viability are affected has not been determined.

Pollen structure

General references on pollen structures include Heslop-Harrison (1971), Stanley and Linskens (1974) and Vasil (1978). Each pollen grain has a thick wall consisting of a very resistant outer exine and an inner intine. The exine begins to form as the microspores separate from the tetrad. It consists largely of sporopollenin which

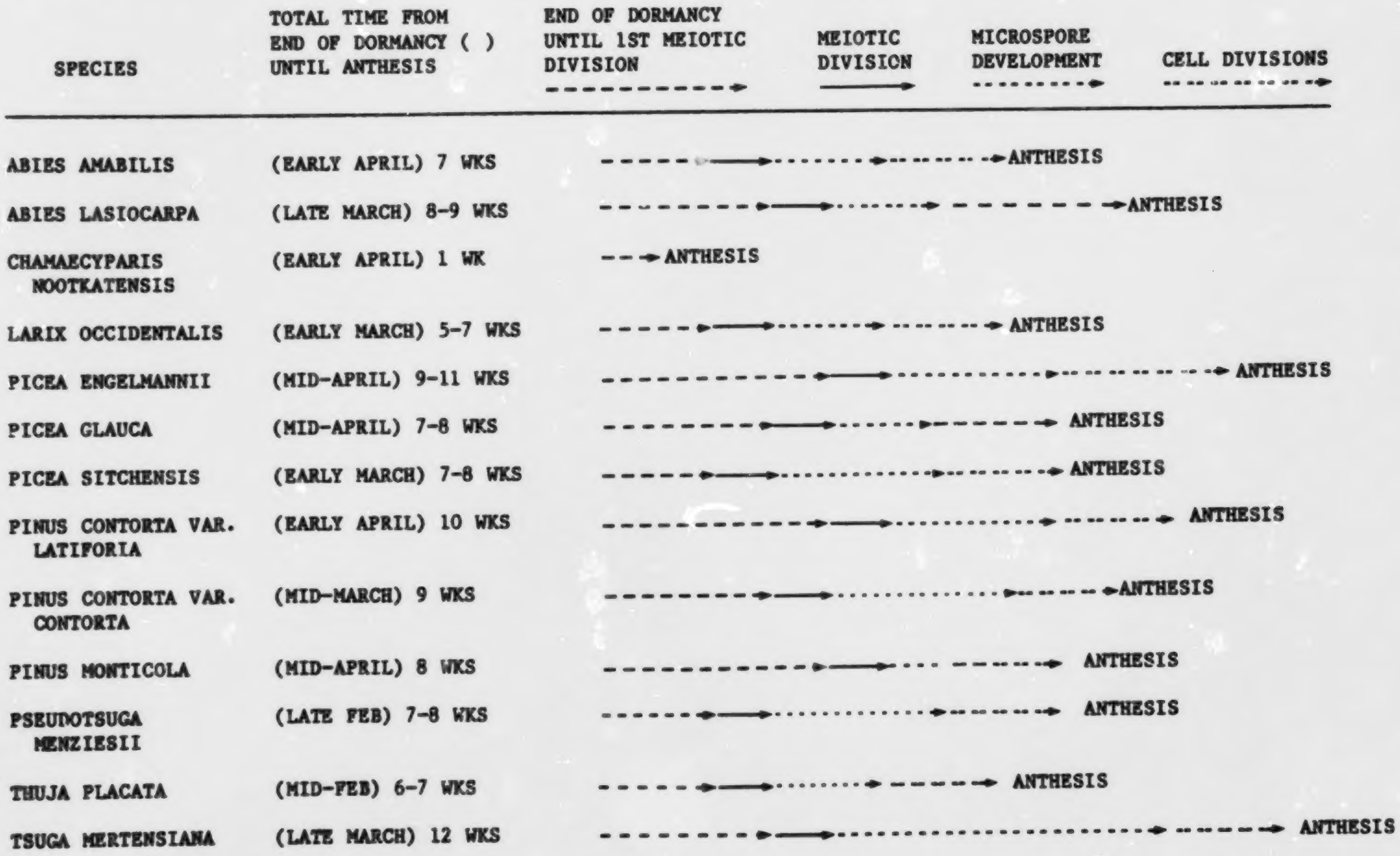


Figure 5.12 Phenology of post-dormancy pollen cone development in 13 native conifers.

renders pollen remarkably resistant to degradation by physiochemical and biological agents and may be variously sculptured. The intine is pectocellulosic and often has cellulose layers laminated with protein. It forms during microspore development and cell division within the pollen grain (Martens and Waterkeyn 1962). The intine eventually forms the pollen tube. Inner layers of the pollen tube wall are of callose. The process of pollen wall formation and the origin of sculpturing in angiosperms are described by Heslop-Harrison (1971). Although there are few such studies for conifers, the process appears to be similar (Vasil 1978). Pollen wall development has been described in Podocarpus (Vasil and Aldrich 1970, 1971) and Pinus (Dickinson 1971, 1976, Willemse 1971c). Sacci development has been described in Podocarpus (Vasil and Aldrich 1970) and Pinus (Dickinson and Bell 1970).

Understanding pollen structure (Singh 1978) and the complex terminology of the palynologist are more important for pollen identification than for understanding seed production. However, pollen identification is important in monitoring pollen flight and contamination in seed orchards and seed production areas. Manuals for pollen identification (Bassett et al. 1978, Owens and Simpson 1982) provide identification only to genus for most forest trees. Variation between species, especially in conifers, is usually too slight to allow easy differentiation (Bagnell 1975).

Pollination biology

The pollination biology of gymnosperms is usually covered with a few paragraphs on wind pollination and brief mention of a pollination drop (Faegri and Van der Pijl 1979). Pollination in angiosperms is the subject around which pollination biology and pollination ecology (anthecology) have developed. The terminology is extensive and basic references should be consulted.

Wind pollination

All conifers and most north temperate hardwood forest trees of commercial value are normally wind pollinated. Yet, the literature on pollination biology deals almost entirely with biotic (mostly insect) pollination. This anomaly results from the fascination of biologists with the complex pollinator-plant interactions, the coevolution of these, and the lack of interest in the more passive process of wind pollination.

Whitehead (1983) has generalized that wind pollination is likely to be successful if certain idealized conditions are met. These include:

(1) production of large numbers of pollen grains;
(2) pollen grains having appropriate aerodynamic characteristics;
(3) flower (cone) and inflorescence structure and location on the plant designed to maximize the probability of pollen entrainment in moving air;
(4) stigmatic surfaces that are structured and positioned to maximize collection efficiency;
(5) pollen release that is timed within both the season and day to maximize possibility of pollination;
(6) relatively close spacing of compatible plants;
(7) a relatively open vegetational structure that minimizes filtration of pollen;
(8) wind velocity within an acceptable range to insure transport and minimize downwind dispersion;
(9) relatively low humidity and low probability of rainfall; and,
(10) unambiguous environmental cues to coordinate flowering.

Wind pollination is an inefficient mechanism, wasteful of pollen, but has the advantage of not relying upon the presence or activity of another organism. Pollen transport by wind involves interactions between the settling (terminal) velocity of pollen and wind velocity. Angiosperm pollen is generally small (20-40 µm), as is that of several conifer families, but in the Pinaceae sizes range up to 100 µm (Owens and Simpson 1982). The density of larger pollen may be decreased by air spaces (sacci), and by dehydration. Small conifer pollen tends not to be saccate. Wind dispersed pollen is seldom ornate (Owens and Simpson 1982). One exception is Tsuga heterophylla; impaction involves spines on the pollen (Fig. 5.8) becoming entangled in cuticular hairs of the bract (Colangeli and Owens 1984).

The importance of filtration was discussed by Tauber (1965, 1967), and later quantified for a forest (Tauber 1977).

Generally, vegetation effectively filters out large amounts of pollen. Deciduous trees are usually not in leaf at pollination. It was shown in four species of Quercus that there was a lag in leaf blade expansion as long as catkins contained pollen. When pollen was dispersed from a tree, there was a spurt in leaf growth (Sharp and Chisman 1961). These authors suggest that arrested leaf expansion facilitates pollen dispersal. Rain also effectively filters out pollen (Tsukada 1982), and fine raindrops scavenge more efficiently than large rain-drops (McDonald 1962). Fortunately, pollen release from wind-pollinated flowers and pollen cones depends on a drying process that usually results in release being timed within the season and day during weather conditions that maximize pollen transfer.

Collection efficiency, the ratio of the number of particles impacting on an object to the number passing through the air space had the object not been there (Tauber 1965), is an important consideration. The probability of impact increases with particle size and density and is inversely proportional to the diameter of the collecting object. That is, smaller surfaces collect more efficiently than large ones. Tauber (1965) demonstrated the effects of collection efficiencies of Betula (21 μm) and Fagus (42 μm) pollen: small light pollen was less likely to be captured than large dense grains, smaller collecting structures have higher collecting efficiencies than larger ones, and the amount of filtration by vegetation increases with wind velocity. The collection efficiencies under many environmental conditions for several plant community structures, and floral parts of various structures and positions, has been predicted (Ogden and Lewis 1960).

Many stigmatic structures maximize pollen collection efficiency. Larger surfaces tend to have thicker boundary layers and hence lower efficiencies than smaller surfaces. Small subdivided surfaces such as feathery or hairy stigmatic surfaces of some flowers and cones are better collectors than flat surfaces. Reduced floral parts, which expose stigmas to wind, and pendulous catkin-like inflorescences (i.e. alder, birch, hickory, aspen, oak, and conifer seed cones) are well designed for pollen capture from moving air (Whitehead 1983). In recent studies using models of fossil seed plants (Niklas 1981, 1982, 1983, Niklas and Norstog 1984) and cones of modern conifers (Niklas and Paw U 1982, 1983, Niklas 1984) in wind tunnels, it was shown that airflow patterns were turbulent around these structures, and maximum impaction of pollen occured on the downwind surfaces. There has evolved an aerodynamic compatibility between the morphologic features of conifer cones and airborne pollen. Detailed studies of airflow patterns around Picea, Larix, and Pinus conelets and cone scales show that pollination is influenced by cone and leaf morphology and the behavior of pollen grains as windborne particles (Niklas 1984).

Settling of pollen onto surfaces may also be affected by electrostatic attraction (Erickson and Buchmann 1983). Plants possess negative surface charges which are greatest near sharp terminal points such as flowers or conelets and least near broad flat surfaces. Cuticular waxes also have excellent dielectric properties when dry. Pollen has a small negative charge when shed, but acquires a strong positive charge as it is carried by the wind. The slowing of pollen movement because of turbulence around a flower or cone (Niklas 1984) could allow settling out, and the probability of settling is increased by selective attraction of oppositely charged particles. Unfortunately, the benefits of electrostatic attraction are largely theoretical and have not been tested on wind-pollinated species. McWilliam (1959b) found no significant bioelectric potential differences between pollen and seed cones of Pinus.

The geographic distribution of plants is associated with pollination. Wind pollination generally increases with latitude and elevation and is dominant in temperate deciduous and boreal forests. Certain environments are appropriate for wind pollination and others are not. For example, the conifer-dominated boreal forests that occur in many high precipitation, mountainous regions are dominated by wind pollinated taxa. Several factors contribute to the high frequency of wind pollination in these areas: close spacing or clumping of compatible trees, the open canopy due to slender pyramidal crowns, positioning of floral structures in tops of crowns, and temporal patterns of rainfall allowing time windows during which wind pollination occurs (Regal 1982, Whitehead 1983). Regal (1982) suggests that wind pollinated species would be selected against in areas of climatic unpredictability.

Although there are many generalizations concerning the ecology of wind pollination, there are few detailed studies. This is particularly true where flower, inflorescence, and cone structure, pollen size and structure, and pollination mechanisms are considered.

Pollen distribution in natural stands

The shedding and dispersal of pollen has been reviewed by Stanley and Kirby (1973). Brief reviews have been published for southern pines (Bramlett 1981) and Pinus sylvestris (Sarvas 1967). The study of pollen dispersal in natural stands involves pollen trapping (Sarvas 1967) or the use of labelled (identifiable) pollen. Buell (1947), using trapping techniques in P. echinata, found that pollen density rapidly dissipated outside the stand compared to that within the stand. Wang et al. (1960) reported that pollen frequency at a distance of 122 m and 152 m was only 2 to 5 per cent of the source frequency. Silen (1962) concluded that only a small fraction of the pollen dispersed by a single open-grown Pseudotsuga tree fell at a distance more than 5 to 10 times the tree height. Tsukada (1982) concluded that a large proportion of Alnus rubra pollen is deposited within a radius of 2 km. Colwell (1951) released radioactive pollen and found that pollen density decreased as distance from the source increased. McElwee (1970) using P^{32}-labelled pollen, found that stand density modified pollen flight, with the majority being deposited within 30.5 to 76.2 m in open stands. All positions of the crown received about equal amounts of pollen released by adjacent trees. Sarvas (1967) also presented data on the vertical distribution of pollen in a stand. Pollen distribution in natural stands is difficult to study but may provide some useful basic information on filled seed production.

Pollen distribution in seed orchards

Studies of pollen distribution are important for seed orchard design and efficient seed production. On young trees just beginning to produce reproductive structures, pollen-cone-bearing often lags behind seed-cone-bearing by several years and strategic placement of known early pollen producers may help avoid pollen shortages. Bramlett (1981) reviews some of the literature dealing with pollen distribution in seed orchards. Furukoshi (1978) made an extensive study of pollination in Cryptomeria seed orchards and discussed many of the problems encountered and alternative solutions which may apply to other species. In an early study, Wang et al. (1960) found that most pollen produced within a seed orchard is deposited in the orchard. Furukoshi (1978) points out that the common practice of topping or hedging may create pollen dispersal problems within the seed orchard by decreasing the height of pollen producers.

Contamination by pollen from trees outside the orchard is often a problem (Franklin 1971). Isolation from or removal of contaminating source trees are possible solutions (Furukoshi 1978). Sprinkling the orchard with water is a commonly used method of delaying conelet receptivity until pollen shed by outside sources has terminated. This is possible, for example, in orchards of high elevation sources where there is already a delay in conelet receptivity compared to local pollen flight. However, Silen (1963) concluded for Pseudotsuga that pollen shed adjacent to orchards may occur for 20 to 30 days and it may be impossible to delay seed cone flower receptivity for this length of time if the phenology of the orchard and adjacent trees are too similar. He calculated that pollen shedding stages progress upslope at a rate of 77 feet per day in Pseudotsuga.

Contamination from outside sources can be a particularly important factor, especially in species where pollen is taken into the ovules on a "first come - first served" basis (Franklin 1971), as in Pseudotsuga (Owens and Simpson 1982). The most important sources of pollen for trees in an orchard are their nearest neighbours (McElwee 1970, Sorensen 1972). More recent studies using gene markers, primarily isozymes, have been useful for determining pollen dispersal and frequency of progeny due to self- and cross-fertilization (Müller 1977, Adams and Joly 1980, Shen et al. 1981). Application of similar isozyme techniques may potentially be used to study optimal time of pollination in seed orchards. Existing gene-marker techniques are of limited value since they only give information about the final result, seed set; they give no information on what has happened between pollination and seed set, especially when low seed set occurs. Methods of estimating pollen contamination and the effect on genetic gain are discussed by Squillace and Long (1981).

Animal pollination

Animal pollination has been studied extensively since the early 1900s and there is a wealth of descriptive, ecological, and experimental literature (see books by Jones and Little 1983, Faegri and van der Pijl 1979). However, few north temperate commercial hardwoods, and no coniferous forest trees, are normally animal pollinated. Detailed studies of insect pollination in hardwood forest trees have been published only for some species of Prunus and Liriodendron (Farmer and Pitcher 1981). Because of the limited relevance to north temperate forest trees, animal pollination (primarily insect) is not reviewed and only some general conditions affecting pollination are mentioned.

Many factors unimportant in wind pollination are significant in animal pollination. Animal pollination is common in plant communities where there is: (1) high species diversity and wide spacing of individuals; (2) absence of a leafless season resulting in high filtration of wind-borne pollen; (3) high humidity and high rainfall probability; (4) absence of unambiguous stimuli to coordinate flowering (uniform temperatures and daylength); and, (5) an abundance of potential animal vectors (Whitehead 1983). These are characteristics of tropical forests where animal pollination is most common. Many of these conditions also exist in some north temperate forests, especially in the understorey of mature stands. Although these conditions are characteristic of animal pollinated communities, they do not preclude wind pollination.

Pollination mechanisms in conifers

'Pollination mechanism' is a term originally used to describe the process of pollen capture and entrance of pollen or pollen tubes into the ovules of conifers (Doyle 1945). This subject has been reviewed by Doyle (1945), Dogra (1964), Konar and Oberoi (1969a), Singh (1978), and Owens (1980). Stigma-pollen interactions in hardwoods would be functionally comparable (Ch. 6). The pollination mechanism is an important consideration in determining the optimal time for pollination and in carrying out controlled or supplemental pollinations.

Modern conifer families evolved over a period of 150 million years (Miller 1977, 1982, Meyen 1984). The ancestral structure of seed cones and ovules suggests pollination by wind and the presence of a pollination drop from each ovule. This is probably the pollination mechanism from which other conifer mechanisms have evolved.

Two pollination drop mechanisms exist. In the Cupressaceae, Taxodiaceae, Taxaceae, and Podocarpaceae, ovules are flask-shaped with a narrow, short neck out of which a pollination drop is exuded (Fig. 5.15). The pollination drop is a clear, dilute solution of various sugars (in the Pinaceae) secreted by the nucellus (McWilliam 1958, J.N. Owens, unpublished results). A typical sequence at pollination has been determined by time-lapse cinematography of Chamaecyparis nootkatensis conelets (Owens et al. 1980). Conelets enlarge and open in the spring, exposing the ovules. In two or three days, pollination drops are exuded from some of the ovules at night but are withdrawn during the day, only to be exuded again the following night. This continues for about two days, then pollination drops are exuded at night and remain during the day for two or three days. Following this, pollination drops are again exuded at night but are withdrawn during the day; finally, they are withdrawn permanently and bract-scales enlarge, burying the ovules within the seed cone. Pollen grains landing on a pollination drop immediately sink into the drop. Pollen grains, landing on the edge of the micropyle before pollination drop formation, are picked up by the pollination drop when it emerges. Similar mechanisms have been described for Callitris (Baird 1953), Thuja (Owens and Molder 1980b), and Taxodium (Vasil and Sahni 1964).

A pollination drop is also exuded in Pinus and Picea but here the ovule is inverted and the ovule tip consists of two micropylar arms (Doyle and O'Leary 1935, McWilliam 1958, Sarvas 1962, 1968, Lill and Sweet 1977, Owens et al. 1981b, Singh and

Owens 1981a, Owens and Blake 1984). Doyle and O'Leary (1935) found that secretion of the pollination drop was a nocturnal phenomenon and that little or no fluid was present during the day. This led McWilliam (1958) to suggest that the pollination drop was produced by guttation, and Lill and Sweet (1977) thought each ovule secreted a drop repeatedly. Recent research on Picea (Owens and Blake 1984, J.N. Owens, unpublished results) shows the drop is secreted by nectary-like tissue at the tip of the nucellus and each ovule secretes a drop only once. Also, it has been shown in Pinus (Owens et al. 1981b) and Picea (Owens and Blake 1984) that micropylar arms secrete minute droplets (Fig. 5.13) to which pollen adheres for several days before a pollination drop forms. Pollen is taken into the micropyle when the pollination drop floods the space between the micropylar arms (Fig. 5.14).

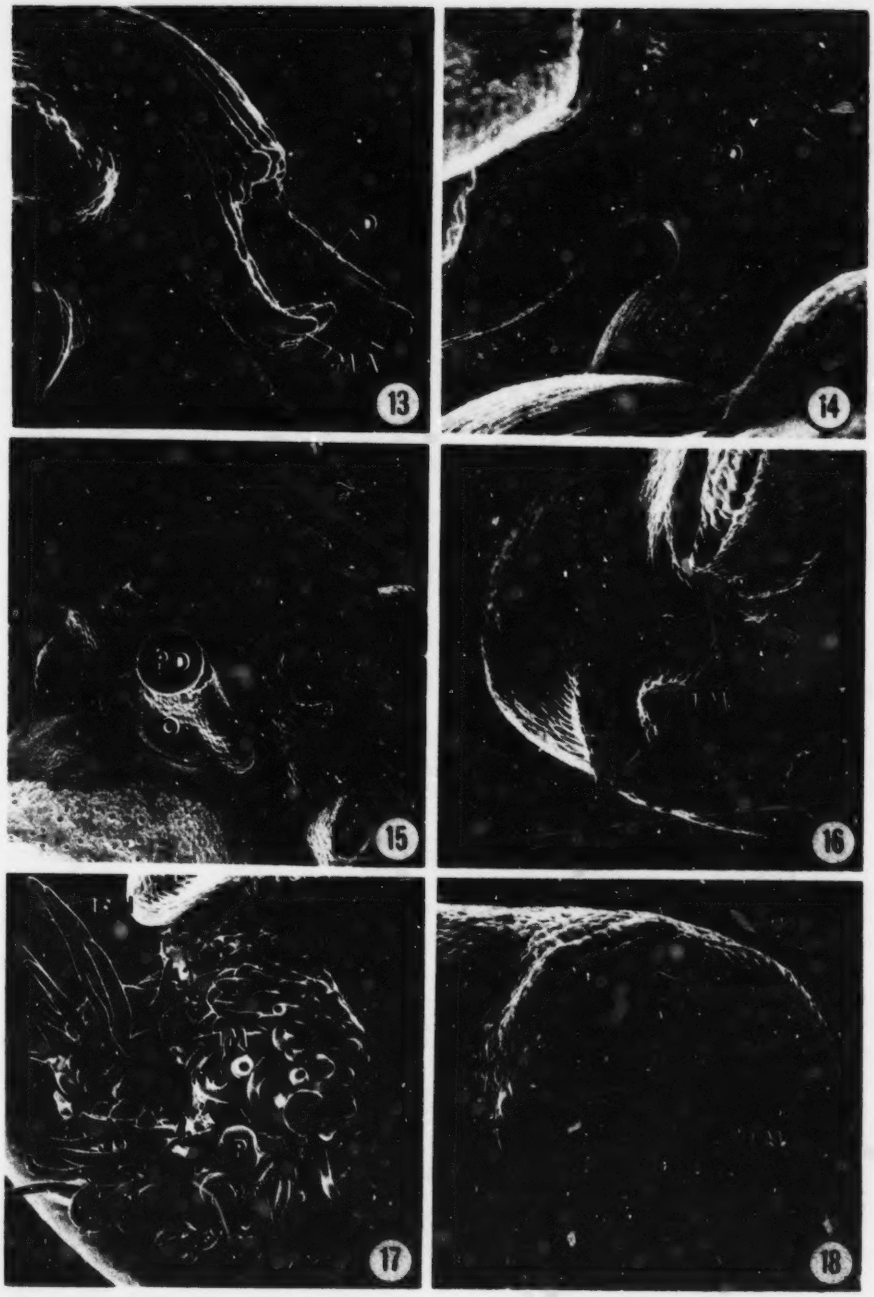
In Abies (Owens and Molder 1977d, Singh and Owens 1981b, 1982) and Cedrus (Doyle 1945) the integument tip is funnel-shaped and somewhat lobed but no pollination drop forms (Fig. 5.16). In Abies (Singh and Owens 1981b, 1982) minute droplets are secreted on the surface of the funnel to which pollen adheres. The funnel then crimps inward carrying pollen closer to the nucellus.

Two pollination mechanisms occur in Tsuga. In the more primitive T. mertensiana the two micropylar arms are broad flaps, similar to that in Picea, which secrete minute droplets to which pollen adheres. The flaps collapse, entrapping pollen grains (Owens and Blake 1983). In T. heterophylla, and probably in most other hemlocks, pollen has spines (Fig. 5.8) which adhere to long web-like cuticular hairs on the abaxial surface of the bract. When the pollen germinates, long pollen tubes are formed which grow into the nucellus (see Ch. 6).

In Pseudotsuga (Allen 1963, Ho 1980, Owens et al. 1981a) and Larix (Owens and Molder 1979c, Villar et al. 1984) the stigmatic tip develops into two unequal lobes covered with unicellular stigmatic hairs, and the micropyle is a narrow slit, too small for pollen to enter. Pollen becomes entangled in the stigmatic hairs (Fig. 5.17). This occurs for several days, then cells around the micropyle collapse and cells on the surface of the lobes elongate, carrying stigmatic hairs and attached pollen into the micropyle. Complete engulfment of stigmatic hairs (Fig. 5.18) occurs within about 2 weeks. There are no secretions on the stigmatic hairs and no pollination drops.

The optimal time for pollination and the duration of effective pollination vary with the mechanism. In species having a pollination drop, most effective pollination occurs when the pollination drop is present (Owens et al. 1981b, Owens and Blake 1984). However, in those that secrete droplets on the micropylar arms, pollen is also effectively collected. Experiments with Pinus (Owens et al. 1981b) and Picea (Owens and Blake 1984) show that some of the pollen which adheres to the arms is taken into the ovule, but the most effective pollination occurs when the ovule has an exuded pollination drop. In these studies, and more recent studies of P. glauca (J.N. Owens, unpublished results), it was shown that not all ovules in a cone exude pollination drops simultaneously. Exudation occurs over several days and progresses acropetally in the cone. Therefore, different regions of each cone are most receptive at different times. This has obvious implications for controlled and supplemental pollinations and implies that maximum seed efficiency (Bramlett et al. 1978) in the field may occur when pollen arrives at the conelets over several days.

In Pseudotsuga pollen is accumulated over several days and the most effective time for pollination is within about 4 days from the time conelets first become receptive (Ho 1980, Owens et al. 1981a). Daniels (1978) showed that wind pollination had a marked cumulative effect until between the seventh and eighth days. His results demonstrate the effectiveness of the pollen collecting mechanism in Pseudotsuga. However, it has since been shown that the first pollen to arrive at the stigmatic surface is taken into the ovule preferentially over pollen arriving later (Owens and Simpson 1982). In Picea glauca that was pollinated at different times with colored pollen, the pollen applied early was most likely to accomplish fertilization (R. Ho, pers. comm.). Lill (1974) suggested that the probability of pollen getting into Pinus ovules is dependent on the proportion of the amount applied to the total present at the time of pollination



Figures 5.13 - 5.18 Scanning electron micrographs of pollination mechanisms. Fig. 5.13. Ovule tip of Pinus showing micropylar arms (MA) with minutes droplets (D). X 110. Fig. 5.14. Ovule tip of Pinus showing the large pollination drop (PD). X 90. Fig. 5.15. Chamaecyparis nootkatensis conelet showing ovules (O) with pollination drops. X 50. Fig. 5.16. Ovule tip of Abies showing open micropyle (M) and funnel-shaped integument tip. X 80. Fig. 5.17. Ovule tip of Pseudotsuga showing stigmatic hairs (SH) with pollen (P) attached. X 80. Fig. 5.18. Ovule tip of Pseudotsuga after the stigmatic tip has been engulfed. X 90.

drop emergence. Somerville and Sweet (1978) have since demonstrated this to be true for P. radiata. Artificial applications of pollen at the right time excluded most natural pollen from the ovule and was responsible for 80 per cent of seed produced.

Other pollination mechanisms which rely on a collection process, as in Abies, Cedrus, and Tsuga, may have long receptive periods possibly without optimal stages. This has been demonstrated in T. heterophylla where each day for more than two weeks, previously unpollinated seed cone flowers were pollinated, but the seed set was essentially the same for all dates (Colangeli and Owens 1984).

Pollination in hardwoods

In angiosperms the capture of pollen relies on certain stigma-pollen interactions. The stigma is the most variable part of the gynoecium. It usually forms a small viscid knob or cleft at the tip of the style but may be more extravagantly developed in some species. The pollen grains adhere to the stigma, due partly to their own stickiness (the oily exine) and partly to the gelatinous or papillate nature of the stigmatic surface (Faegri and van der Pijl 1979). The stigmatic surface may be dry at maturity, having a dehydrated proteinacious extracellular layer or pellicle but no free secretion, or wet, having a similar but hydrated layer. The surface may be smooth or have unicellular or multicellular papillae. Families containing hardwood forest trees are fairly homogeneous in stigma type, either papillate or non-papillate and usually with a dry surface (Heslop-Harrison and Shivanna 1977).

In wind pollinated angiosperms the capture of pollen by the stigma may be facilitated by its exposure outside the floral envelope and its large, often sticky surface (Heslop-Harrison and Shivanna 1977, Ager and Guries 1982). Sticky stigmatic surfaces occur in Ulmus (Ager and Guries 1982), Quercus (Kolpak et al. 1980), Juglans (Germain et al. 1973), and others (Fechner 1979). Wind pollinated plants are frequently characterized by long filaments which bring the anthers outside the floral envelope to disperse pollen in the air currents. In others (e.g. Betula, Corylus), pollen grains lodge between closely fitting catkin scales and when the catkin moves in the wind the scales open and pollen is dispersed (Faegri and van der Pijl 1979).

Synchrony and time of flowering

One characteristic showing considerable variation between and within species is the time of male and female flowering. Klaehn (1961) suggested that in conifers, conelets commonly become receptive before pollen cones on the same tree shed their pollen. However, there are too many exceptions for this to be a general rule. A similar trend may occur in hardwoods. Several studies have shown that tree species are seldom entirely protandric (male structures mature before female) or metandric but populations vary in frequency of each. Early studies of Picea abies suggested that this resulted from differential responses of pollen and seed cone buds to warm spring temperatures (Meehan 1888). It has since been shown that differences in time of male and female flowering may vary within a population in different years (Chung 1981).

The time of flowering of any species varies because of inherent differences among individuals and because of the range in latitude and elevation over which it is distributed (Fowells 1965, Snyder and Clausen 1974). Despite variation in overwintering stages (Fig. 5.1), rates of pollen development (Fig. 5.12) (Owens 1980), and, the as yet poorly understood dormancy requirements for reproductive buds, there is considerable overlap in pollen shed and conelet receptivity within most conifers. Chung (1981) provides an extensive discussion and review dealing with factors affecting flowering time and the implication this has for natural populations and seed orchards. Synchrony has been observed in Pinus (Wright 1953, Bramlett 1973, Grano 1973, Beers 1974, Barnes and Mullin 1974), Abies (Franklin and Ritchie 1970), and several other genera within the Pinaceae and Cupressaceae (Owens 1982). In conifers, asynchronous development is not commonly a major barrier to self-pollination.

In wind pollinated hardwoods there is little information on synchrony. Information on several genera is summarized by Farmer and Pitcher (1981). In some monoecious species pollen dehiscence and female receptivity in the same flower are not synchronized. Fraxinus and Populus are metandrous and Plantanus is protandrous, whereas this is unspecified in other genera such as Quercus. In some genera, such as Liriodendron, male and female flowering overlap and occur over very long periods of time.

Wind pollinated forest trees produce large amounts of pollen. Conifers are particularly prodigious pollen producers, often creating "sulfur showers" at peak pollination. Estimates of pollen production are important in seed orchards. The number of pollen grains produced per pollen sac or cone has been measured for several species. The amount of pollen per cone depends on the size of pollen and microsporangia and the number of microsporangia per pollen cone. Smaller pollen cones, as in Tsuga heterophylla, may produce about 50,000 pollen grains (Ho and Owens 1974a) but those with very small pollen grains such as Chamaecyparis obtusa contain about 198,000 (Saito and Takeoka 1983). The intermediate sized pollen cones of Larix leptolepis (Yokoyama et al. 1978) and Pinus contorta (Ho and Owens 1974c) produced 91,000 and 465,000 respectively while the largest pollen cones in Araucaria may produce 10 million pollen grains (Chamberlain 1935). Considerable variation occurs in estimates of pollen production. Adams (1982) found the number of pollen grains per pollen cones of Pseudotsuga varied from 37,310 to 62,960 among eight seed orchard clones. This was comparable to results of Orr-Ewing (1965), and Ho and Owens (1974b), but not results obtained by Sziklai (1963). Electronic counters appear to give the most consistent results for pollen counts. Simple yet accurate methods should be developed for each species to estimate pollen production based on sample branches and trees in seed orchards. Estimates have not been made for hardwood forest trees.

The proportion of pollen produced that reaches the conelet or flower in both wind and insect pollinated trees is unknown but represents only a minute fraction of that produced (Stephenson and Bertin 1983).

The onset, duration, and rate of pollen release are temperature dependent. Most north temperate forest trees appear to have a cold requirement (Pharis et al. 1969, Simak et al. 1974, Katsuta 1975). However, some southern pines do not. With favourable temperatures, pollen cones of Pinus clausa may shed pollen in late November or in December (Boyer 1981). In contrast, P. taeda has a dormant period and will not resume development without chilling (2000 hrs at 4°C). In hardwoods, there also appears to be a cold requirement before floral development will resume. Several Populus species and hybrids have variable chilling requirements and it appears that southern ecotypes of some forest trees require less total cold exposure than do northern ones (Farmer 1964). Physiological changes occurring in floral buds during chilling have not been studied in forest trees and have only been studied to a limited extent in fruit trees (Felker et al. 1983).

Heat accumulation following the chilling period has been used to predict onset of flowering in Pinus (Sarvas 1962, Boyer 1973, 1978, 1981) and Picea (Sarvas 1968). Other studies have followed the phenology of species and correlated onset of spring bud activity with minimum temperatures. In a study of 19 species there were no obvious correlations between current temperature levels and flowering or leafing, and considerable variation occurred from year to year (Ahlgren 1957). Boyer (1981) discussed in detail the use of temperature and the alternatives of using degree-hour or degree-day sums to predict pollen release.

The duration of pollen release is highly variable in a species from year to year and is related to daily fluctuations in temperature and humidity. Pollen shed in coastal British Columbia has peaks during the day (1000 hr to 1600 hr) and although pollen may be shed over many days or weeks there are also peak periods during this time (Ebell and Schmidt 1964). In southern pines the duration of pollen shed is arbitrarily set as the fewest consecutive days needed for dispersal of 80 per cent of the total pollen. In Pinus palustris dispersal took as few as 5 days and as many as 21 days and averaged 13 days over 22 years (Boyer 1981).

Controlled and supplemental pollinations

Controlled and supplemental mass pollinations (SMP) are commonly used in tree breeding and in seed production in conifers and to a lesser extent in hardwood forest trees. Controlled pollination and SMP must be done at the optimal time for maximum seed set. Several studies have been made of conifers to determine the optimal time of pollination. These combine careful phenological studies of conelet development with controlled pollinations done at different times. Most studies correlate time of pollination with seed efficiency at the end of the growing season (Bramlett et al. 1978). Other studies have correlated time of pollination with amount of pollen taken into the ovule, as well as seed efficiency (Yokoyama et al. 1973, Somerville and Sweet 1978, Ho 1980, Owens et al. 1981a, 1982, Owens and Simpson 1982, Owens and Blake 1984). The latter technique, though more difficult, provides morphological information about causes of failure to set seed. In all cases, phenological studies must identify recognizable stages of conelet development and not rely upon calendar dates.

One of the earliest studies of controlled pollinations in which phenological stages were carefully monitored was of Pinus (Cumming and Righter 1948). These same stages are still used for controlled pollinations in southern pines (Bramlett and O'Gwynn 1981). There have been several subsequent reports on pollination techniques for Pinus (Wakeley and Campbell 1954, Mergen et al. 1955, Lill 1974, Somerville and Sweet 1978) and other conifers including Tsuga (Nienstaedt and Kriebel 1955), Pseudotsuga (Orr-Ewing 1956, Carlson and Hsin 1976, Daniels 1978) and Picea (Owens and Blake 1984).

The various methods of pollen application in controlled pollinations are described by Bramlett and O'Gwynn (1981) and Matthews and Bramlett (1981). Specific recommendations are made which apply generally to conifers. SMP is a method of broadcast application of pollen to conelets that are not isolated from airborne pollen. This method is used to increase seed yield in orchards and seed production areas. In many situations, especially young orchards, airborne pollen is too limited for adequate seed set. In Pinus adequate pollen is essential or conelets abort (Sarvas 1962, Sweet 1973), whereas in most other genera conelets develop but seed efficiency may be very low (Owens et al. 1981a). Other reasons to use SMP include: introduction of specific pollen lots, compensation for poor synchronization or low production of local pollen lots, minimization of the effects of poor weather conditions at the time of natural pollen shed, dilution of the effects of external pollen sources, increased genetic gain by panmixia among orchard clones, and production of interspecific hybrids (Bridgwater and Trew 1981). Recommendations for SMP and equipment used are also described by Bridgwater and Trew (1981). Daniels (1978) concluded that SMP clearly has potential as a method of increasing the quantity and genetic quality of seed in orchards. Increased seed yield by SMP has been demonstrated using various SMP techniques in Pseudotsuga (J.E. Webber, pers. comm.) and Pinus (Hadders 1977, Bridgwater and Trew 1981) seed orchards.

Controlled pollination and, especially, SMP may never be as extensively used in hardwoods as in conifers (Farmer and Pitcher 1981) because of the complexity of the pollination process and difficulty in collecting large quantities of pollen in many hardwoods. Farmer and Pitcher (1981) describe pollination techniques for Fraxinus and Liquidambar and give background information on Prunus, Juglans, Populus, Quercus, Plantanus, and Liriodendron. Techniques for controlled pollinations are described for several genera including Salix (Argus 1974), Populus (Knox et al. 1972b), Juglans (Beineke and Masters 1976), Prunus (Forbes 1973), Quercus (Ledig et al. 1971), Alnus, (White 1981) and Eucalyptus (Van Wyk 1981).

Pollen management

In order to carry out controlled pollination and SMP it is necessary to collect and store pollen. This requires knowledge not only of the phenology of pollen development in each species but of methods of pollen collection, storage, viability testing and pollen physiology. Pollen collection, storage, and viability testing were thoroughly reviewed recently under headings of "pollen handling" (Snyder and Clausen 1974) and "pollen management" (Franklin 1981). Pollen storage, viability testing, and physiology were reviewed by Johri and Vasil (1961), Rosen (1968), and Binder et al. (1974). Our purpose is to update these reviews on aspects most relevant to seed production in forest trees.

Pollen collection

Pollen collection techniques must take into consideration the phenology of pollen cone development so that collections are made at the proper time for maximum pollen yield and viability. This necessitates monitoring trees and collecting on a tree by tree or even branch by branch basis. Procedures may be unique to each species and location. They involve the collection of nearly mature pollen cones and extraction of pollen in the laboratory, the bagging of branches or trees to extract pollen in the field, or forcing pollen cone development on cut branches or trees.

Seitz (1958) described the autumn collection of predormant male branches of aspen and the subsequent chilling and forcing to produce normal pollen. Beers et al. (1981) described methods of sampling for cone maturity, forcing techniques, and extraction for Pinus taeda and P. elliottii. Kudo (1980) followed pollen cone development and determined the most favorable time for pollen collection in species of Abies, Picea, and Pinus, but sample sizes were small and variation was high. Snyder and Clausen (1974) described methods of pollen collection. They emphasized conditions for short-term forcing from cut branches, the importance of correct timing of collections, and the quantity of pollen cones or flowers needed from different species to obtain particular volumes of pollen. Useful tables are provided and they emphasize that dates of flowering for a species are handy guidelines but exact timing will vary with locality, year, and individual tree. They reviewed much of the literature dealing with forcing pollen on cuttings from hardwood forest species. Dates of flowering for different species are provided by Fowells (1965) and Schopmeyer (1974).

Pollen storage

In general, pollen that is properly collected, extracted, and dried will store well, whereas even the best techniques will not allow storage of poorly handled pollen. Generally, the longevity of stored pollen increases with decreasing moisture content, but there are exceptions (Stanley and Linskens 1974). The best extraction techniques involve some humidity control to reduce the moisture content of pollen (Sprague and Snyder 1981). Pollen may need further drying after extraction (Snyder and Clausen 1974). Freeze-drying followed by deep freezing is an effective technique for extending the period of pollen storage (Duffield and Callaham 1959, Ching and Ching 1964, King 1965, Livingston and Ching 1967, Ching 1969, Ichikawa and Shidei 1971, 1972a, b). Pinus monticola (Ching and Ching 1964) and Pseudotsuga (Livingston and Ching 1967) pollen was freeze dried then stored. Air drying, and cold storage prior to freeze drying, both improved the quality of the freeze dried pollen. Pollen of several tree species has been stored at temperatures varying from +5° to -23°C and relative humidity varying from 0 to 50 per cent for a few months to 13 years, depending upon species and initial quality of the pollen (Barber and Stewart 1957, Duffield and Callaham 1959, Ehrenberg 1960, King 1965, Callaham and Steinhoff 1966, Bingham and Wise 1968, Alam and Grant 1971, Bingham et al. 1971, Popnikola 1971).

Generally, conifer pollen is easier to handle and stores longer than angiosperm pollen (Stanley and Linskens 1974). The storage period for viable angiosperm pollen is usually measured in days, whereas that of conifers is measured in months or years. Recommendations for short and long term storage of pine pollen are described by Matthews and Kraus (1981). Conditions for conifer pollen storage are given by Snyder and Clausen (1974). Extensive research on pine pollen (Sprague and Johnson 1977) shows that a low (8 to 10 per cent) initial moisture content was the most important factor for successful storage. Also vacuum storage was better than storage with no vacuum. Recommendations for storage of angiosperm pollen, including several hardwood forest trees, are reviewed and tabulated by Snyder and Clausen (1974). Quercus and Juglans pollens have been effectively stored in liquid nitrogen (Makhmet and Shlonchak 1977).

Binder et al. (1974) reviewed pollen storage with an emphasis on gymnosperm pollen. They concluded that the respiration of pollen must be reduced to conserve its food reserves and that temperature and humidity of stored samples were the most important variables in longevity of pollen in storage. Wang (1975) suggested that "Possible causes of pollen deterioration in storage are: (1) exhaustion of respiratory substrate, (2) inactivation of enzymes, growth hormones, and pantothenic acid, (3) desiccation injury, (4) accumulation of secondary metabolic products, and (5) changes in lipids of the exine of the pollen membrane and lipid autoxidation..."

Some research has been done on structural and physiological changes during pollen storage. When conifer and angiosperm pollen was deep-frozen, mechanical injury included pseudoplasmolysis, ice crystal and air bubble formation, and surface cracks. Physiological injury included decreased or complete loss of ability to form starch and pollen tubes (Ichikawa et al. 1970; Ichikawa and Shidei 1971, 1972a, b). Loss of Populus pollen germinability depends on the appearance of one or more inhibitory protein-like substances (Dhir et al. 1982). Bingham et al. (1964) have also demonstrated increased denatured protein in poorly stored pine pollen as shown by increased protein bands in elecrophoretic patterns. Pseudotsuga pollen under adverse storage conditions showed a decreased respiration rate (Binder and Ballantyne 1975). Another effect appears to be membrane damage as measured by conductivity of pollen leachate (Ching and Ching 1964, 1972). Although certain types of storage adversely affect pollen viability, there is not yet a clear picture of all the structural or physiological changes that occur. A recent study suggests that selection against weaker pollen may occur during storage, resulting in stronger seedlings from stored pollen (Mulcahy et al. 1982).

Pollen testing

Pollen viability or quality, usually as it relates to storage, was reviewed by Visser (1955), Binder et al. (1974), Snyder and Clausen (1974), Stanley and Linskens (1974), Goddard and Matthews (1981) and Heslop-Harrison et al. (1984). Pollen viability was originally based on germination tests and seed or fruit set. Pollen germinability is the ability to produce pollen tubes under what are assumed to be optimal in vitro growing conditions and pollen fertility is the ability to produce normal seed (Jensen 1964). Obviously, all pollen that germinates may not have the vitality to survive or compete and take part in fertilization. Therefore, germinability may not be an adequate measure of pollen "viability". Jensen (1964) showed that fertile pollen invariably showed positive germinability but the reverse was not necessarily true. Similarily, Duffield (1954) stated that germination tests are not a reliable assessment of the fertilizing potential of pollen. Therefore, pollen viability tests which also give some measure of vigor are very important.

Tests of pollen quality have been used extensively (Stanley and Linskens 1974, Heslop-Harrison et al. 1984). Snyder and Clausen (1974) described procedures and tabulated methods and results for many angiosperms including most hardwood forest genera. They gave similar but more limited procedures for several conifer genera, mostly from the Pinaceae. Goddard and Matthews (1981) provided detailed procedures for pine pollen and emphasized the need for standardized procedures which give verifiable results. A few studies have tested effects of various minerals, growth substances, and sugars on pollen tube growth (Echols and Mergen 1956, Dillon and Zobel 1957, McWilliam 1960, Vasil 1960, Ho and Sziklai 1971a, b, 1972).

Pollen germination tests made before 1932, when boron was discovered to be an important stimulant to germination, are of questionable value (Stanley and Linskens 1974). Early tests of forest trees involved broad surveys of media and conditions (Echols and Mergen 1956). Several types of germination tests are described by Stanley and Linskens (1974), but they may be most applicable to angiosperm pollen, where germination is generally rapid and easily identified pollen tubes develop. Germination is generally slower in conifers (Snyder and Clausen 1974) and, in some genera (Pseudotsuga, Larix) pollen elongates (Ho and Rouse 1970, Ho and Sziklai 1972a, Allen and Owens 1972) but does not form a pollen tube until it reaches the nucellus several weeks later (Allen and Owens 1972, Owens and Molder 1979c). It is difficult to distinguish between swelling and elongation and active pollen tube growth. The extent of pollen tube growth necessary to indicate "germination" is also difficult to determine even in species having typical pollen tube development. Pollen tube growth may decrease with increasing pollen age (Kuhlwein and Anhaeusser 1951) and storage time (Cram and Lindquist 1984), which supports Duffield's (1954) and Dempsey's (1962) suggestions that rate of pollen tube growth should be employed as a measure of fertilizing potential. Because of the frequent ambiguous results, especially with conifers, and the need for reasonably precise techniques and equipment, unavailable in some laboratories, other techniques for accessing pollen quality have been developed. However, germination tests will no doubt remain a useful standard technique.

Staining techniques, like germination tests, have been used to estimate pollen viability. Binder et al. (1974) reviewed the literature of the 1950s and 1960s and concluded that none of the staining tests were generally reliable. Snyder and Clausen (1974) and Goddard and Matthews (1981) also discussed staining methods and concluded that techniques are not widely accepted and are generally unsatisfactory indicators of viability. Some stains, like acetocarmine or safranin, are histological stains which give no indication of physiological viability. Other "vital" stains, such as nitro blue tetrazolium (Nitro-BT) (Hauser and Morrison 1964), are based on the oxidative metabolism of living pollen. With many staining techniques the colour reaction is influenced not only by viability but also by temperature, time of staining, and species of pollen. Response to tests can be judged to fall into other categories besides living or dead. Intermediate categories may represent varying degrees of vigor or degeneration. Many staining tests are useful, but only if parallel tests of seed set are run using the same batches of pollen. Technicians working with one species can frequently develop a reliable staining test for their situation. However, this test cannot always be transferred to another species or used by other technicians without repeating the tedious seed set comparison.

Pollen viability has been tested in forest trees using tetrazolium salts such as 2,3,5-triphenyltetrazolium chloride (TTC) (Cook and Stanley 1960, Chira 1963, Chen 1981) and Nitro-BT (Hauser and Morrison 1964). A peroxidase reaction was used in Larix, Pinus, Pseudotsuga, and several angiosperm species (Maurinj and Kaurov 1956). Methyl green and phloxine have been used for testing Larix and Betula (Worsley 1959). Fluorochromatic dyes (Ryynanen 1978) and acetocarmine have also been successfully used (Heslop-Harrison and Heslop-Harrison 1970, Heslop-Harrison et al. 1984), as well as potassium idodide (Jovancevic 1962). In a comprehensive study of angiosperm pollen quality testing methods, Heslop-Harrison et al. (1984) concluded that the fluorochromatic reaction, a histochemical procedure which tests for the presence of an active esterase and integrity of the plasmalemma, correlates highly with in vitro germination methods. The speed of most other staining tests is outweighed by low reliability and repeatability. As a result, other rapid techniques based on pollen physiology have been developed.

Tests of electrical conductance and leachates are based on the premise that, as pollen ages, membranes change and substances leach out in water. Ching and Ching (1976) placed Abies, Pseudotsuga, and Tsuga pollen in distilled water and measured the leachate by ultraviolet absorption, sugar and amino acid content, and elecrical conductance. High values for all these were closely related to germination tests of the same pollen lots. Similar results were obtained using Pinus taeda pollen (Foster and Bridgwater 1979). A chemical assay of pine pollen showed the concentration of low molecular weight sugars and organic acids to be higher in viable than in non-viable pollen and this was related to in vitro germination capacity (Stanley and Poostchi 1962). Goddard and Matthews (1981) describe a procedure for measuring electrical conductance of many pollen lots simultaneously. They obained high correlations between this test and germinability of P. elliottii pollen and concluded that pollen deterioration is accompanied by loss of membrane integrity. The amount of leachate may also indicate the relative vigor of pollen.

Respiration of pollen from forest trees was studied by Livingston (1971b), Hygaard (1973) and Binder and Ballantyne (1975). In the last study, the respiration rate of Pseudotsuga pollen was measured using a Clarke oxygen electrode and compared to the number of filled seed. They concluded that there was a positive correlation between pollen respiration and pollen fertility. Ching et al. (1975) found the content of adenosine triphosphate (ATP), which provides energy for biosynthesis and growth, was significantly correlated with germinability of Abies, Pseudotsuga, and Tsuga pollen. Although both of the above tests require only small pollen samples and a few minutes to perform, they require some specialized equipment. This equipment is not difficult to operate but is expensive. These techniques probably provide the most unambiguous results because they are based on quantitatively measurable metabolic processes. More recently, cyclic AMP and GMP have been shown to have some physiological or biochemical regulatory effects on germination of *Pinus densiflora* pollen but this was not suggested as a viability test (Katsumata et al. 1978).

Pollen physiology

There are complete reviews of pollen physiology (Johri and Vasil 1961), physiology with emphasis on gymnosperms (Binder et al. 1974), and ultrastructure and physiology (Rosen 1968). The first two deal with the physiology of pollen in storage and culture, viability tests, and pollen chemistry. Pollen chemistry considers sugars, proteins and amino acids, vitamins, growth promoters and inhibitors, enzymes, isoenzymes, metabolism, and respiration. Rosen (1968) reviewed pollen in a very broad sense, including the above aspects as well as ultrastructure and incompatability reactions. Understanding pollen physiology is important in developing methods for pollen extraction, storage, and viability testing. It is also essential for understanding changes which occur during pollen tube growth in vivo, and interactions between pollen tubes and stigma, style or nucellar tissues (Ch. 6).

Carbohydrates not only are major constituents of the pollen wall (Heslop-Harrison 1975) but may be abundant within pollen as free sugars or starch. Mameli (1952) generalized incorrectly that wind-pollinated plants have starchy pollen while entomophyllous plants have pollen rich in fat and sugar. In conifers, the Cupressaceae have oil-rich pollen (Owens et al. 1980) and the Pinaceae have starch-filled pollen (Owens and Molder 1971a). Also, Johri and Vasil (1961) state, "At various stages of development, pollen grains show considerable quantities of starch but invariably it disappears during cell division or at the time of shedding..." This is not true for the Pinaceae, where considerable amounts of starch are present in pollen tubes while they grow through the nucellus (J.N. Owens, unpublished results). The major component of free sugar in pollen varies with species. In angiosperms, sucrose comprised 20-50 per cent of free sugars, whereas in pines it may be more than 93 per cent (Stanley 1971). Most pollens contain many soluble sugars. In pollen from 15 conifers, stachyose occurred in 10, arabinose, xylose, and galactose occurred frequently, often as hydroxylates of pectin and hemicellulose (Stanley 1971). Chira and Berta (1978/79) identified 13 sugars in pollen from 25 taxa of *Pinus* and from seven species of *Abies*. In pine pollen, soluble sugars decreased much more rapidly under poor than under favorable storage conditions (Stanley and Poostchi 1962).

The total protein in pollen is generally between 11 and 30 per cent (Stanley 1971). Protein synthesis increases at germination (Mascarhenas and Bell 1969) and protein levels are higher in fast growing than in slow growing pollen tubes (Stanley 1971). *Betula* pollen showed a considerable change in protein composition with age. All protein studies of forest trees appear to have been done on ungerminated pollen or pollen grown in vitro.

Vitamin content of gymnosperm pollen is usually low, whereas anigosperm pollen is rich in B-vitamins but poor in fat-soluble vitamins (Lunden 1954). High levels of vitamins, sugars and fats in angiosperm pollen may be related to insect pollination since pollen is a food source of many insect pollinators.

Plant growth substances have been identified in conifer pollen (Michalski 1967). Thirteen growth regulators, including three inhibitors, several auxins, gibberellins (GAs), and possibly cytokinins, were identified in *Pinus radiata* pollen (Sweet and Lewis 1969, 1971). Ga_3, GA_4, and GA_7 have been characterized in *P. attenuata* pollen (Kamienska and Pharis 1975, Kamienska et al. 1976a). Also, GA activity changed from predominantly non-polar GAs in dormant pollen to more-polar GAs as germination progressd in *P. attenuata*, *P. coulteri*, and *P. ponderosa* (Kamienska et al. 1976b). Sweet and Lewis (1971) demonstrated that one GA, when extracted and reapplied, enhanced pollen tube growth. Research using angiosperm pollen implicates auxins, GAs, and cytokinins in germination and pollen tube growth (Barendse et al. 1970). Effects of various growth regulators have been tested on *P. roxburghii* pollen, and their enhancement of growth was manifested through cyclic AMP; phytochrome was also involved (Dhawan and Malik 1981). Although pollen appears to possess a simple system in which to study the primary effects of growth regulators, the physiology is complex.

Generally, pollen has enzyme activities similar to other plant structures (Binder et al. 1974). Most studies of forest tree enzymes center around isoenzymes used as genetic markers. Although isozymes have been identified in several species of angiosperm pollen (see Binder et al. 1974), isozyme studies in conifers generally utilize the haploid female gametophyte tissue rather than pollen and compare the former to the diploid embryo (sporophyte) tissue in the seed (Lewis 1981).

Several factors may affect pollen germination. High doses of radiation (above 100kR) depress or prevent pollen tube growth, whereas lower doses may stimulate pollen tube growth (Mergen and Johansen 1963, Fujimoto et al. 1964, Clausen 1973b, Livingston and Stettler 1973). Livingston (1971b) found low radiation increased growth because of accelerated metabolism, whereas decreased growth at higher doses may have resulted from changes in cell wall or membrane structure. High doses of radiation have been used to inhibit germination and produce mentor pollen in Populus (Stettler 1968).

Atmospheric sulfur dioxide (SO_2) at concentrations above 0.75 ppm reduced germination of moist Populus pollen and concentrations of 1.4 ppm reduced germination and pollen tube elongation in moist Pinus and Picea pollen grown in vitro (Karnosky and Stairs 1974). Their results showed that the primary effect of SO_2 exposure may be correlated with absorption of SO_2 by the germinating media. Additional work is needed to test the applicability of in vitro studies to in vivo conditions. It is possible that stigmatic and pollination drop fluids could be toxified by SO_2 absorption. The SO_2 concentrations which decreased conifer pollen germination and pollen tube elongation in vitro are within the range of reported concentrations in areas of significantly decreased P. ponderosa cone production (Scheffer and Hedgecock 1955). P. strobus pollen exposed to ozone (O_3) showed no adverse effect if pollen was dry. However, O_3 fumigation of wet pollen significantly reduced per cent germination but did not inhibit pollen tube growth in the unaffected pollen (Benoit et al. 1983). Sidhu (1983) found no adverse effects of acid rain on Picea glauca pollen if pH was above 3.6 but germination was reduced by up to 30 per cent at a pH equal to or less than 3.6. The limited observations on effects of air pollutants on conifer pollen suggest that pollination mechanisms involving a pollination drop or conditions which otherwise hydrate exposed pollen may increase the adverse effects of the pollutant.

Summary, and recommendations for future research

The phenology of meiosis, pollen development, and anthesis are extremely variable in forest trees. Species grown outside their natural range may vary from those within the natural range and the former may undergo meiotic or pollen abnormalities or pollination irregularities. With the exception of floral initiation, pollination is probably the most important step and weakest link in the reproductive cycle. However, specific causes of poor pollination are numerous and may vary with species and location. Identifying the causes could significantly increase seed production in orchards and seed production areas; however, this would have little impact on improving seed production in natural stands.

Studies to determine the most effective time for controlled pollination or SMP are essential but have been done for very few species. Techniques used involve phenological observations and the precise timing of pollination, followed by dissection, counts of pollen taken in, and estimation of seed efficiencies. Simple methods using stained pollen and genetic markers (e.g. isozymes) are useful. However. before extensive field studies are done, the pollination mechanism should be understood for the species.

Although the general conditions favoring wind pollination are understood, little is known of how these apply to different species in natural stands or seed orchards. Even less is known about insect pollinations in hardwood forest trees. Such studies serve as essential background for determining procedures to increase seed production.

Studies determining the optimal time, method, and benefit of SMP and the effect of early arriving pollen or pollen contamination in orchards, are required. The use of controlled pollination and SMP will increase as more seed orchards are established. This will require detailed information on pollen management procedures such as pollen forcing, collection, extraction, storage, and viability testing. We know little about the effect of forcing on pollen viability and vigor. Storage and viability testing should be done for each species because of the structural and physiological differences between pollen from different species. Methods should be developed to determine pollen vigor in addition to viability, because success in fertilization may depend upon slight, as yet unmeasured, differences in vigor. Tests of vigor and viability should be correlated with seed efficiency using the same pollen lot. The lower limits of pollen vigor which allow adequate seed set should be determined.

Standardized procedures for pollen viability and vigor tests should be developed for each species. Germination tests may work for some species but growth rate data are essential in determining vigor. Physiological tests involving electrical conductance, and respiratory and ATP levels, are promising because they have a known physiological basis which relates to vigor. Fluorochromatic tests show promise as quick, inexpensive, and reliable estimates of pollen quality but more tests need to be made on pollen from forest trees. Basic changes in pollen metabolism, chemistry, and ultrastructure during pollen storage, hydration, and germination should be investigated further in order to develop optimal storage conditions for maintaining maximum pollen vigor.

Studies of the effects of environmental pollutants on pollen vigor should determine the upper allowable limits of pollutants. This may be most important in species which have pollen that remains hydrated and exposed to the atmosphere for prolonged periods.

CHAPTER 6

GAMETOPHYTE DEVELOPMENT AND FERTILIZATION

Introduction

The male gametophyte is the small haploid multicellular structure that develops from the pollen grain and within which the male gametes develop. The female gametophyte is the large multicellular structure in which the egg(s) form within the ovule. The present subject thus includes pollen germination, pollen tube development, penetration of the ovule, and prefertilization ovule development. Fertilization specifically involves the release of male gametes and their fusion with female gametes. In many references the term "fertilization" is used more generally, and includes pollen germination and pollen tube growth as well as prefertilization ovule development. Male gametophyte development in conifers and angiosperms differs in rather subtle ways, whereas female gametophyte development and fertilization are quite different in the two groups. These differences are important in understanding seed development. Terminology also differs somewhat between conifers and angiosperms. The correct botanical terms for both will be used here because they show differences in origin, development, and function of structures. Singh (1978), Foster and Gifford (1974), and Kozlowski (1971) are useful references on these topics. There have been no reviews of this subject for forest trees but a recent book "Mate Choice in Plants" (Willson and Burley 1983) discusses some of the relevant literature.

Post-pollination male gametophyte, development in conifers

The site of pollen germination

Variations in location of pollen at germination, and in pollen tube development, are related to the pollination mechanisms described in Chapter 5. In members of the Pinaceae having a pollination drop (Picea, Pinus) the top of the nucellus secretes the drop. Pollen is withdrawn into the ovule with the drop (Fig. 5.15) and may settle into a depression (pollen chamber) in the tip of the nucellus. The chamber, at least in Picea, develops by differential cell division and enlargement rather than collapse of cells (Owens and Molder 1979a, Singh and Owens 1981a, Owens and Blake 1984). Development of the pollen chamber has not been described in Pinus (Sarvas 1968). Chambers vary in size in different conifers. Willson and Burley (1983) suggest that large chambers increase the probability of deposition of outcross pollen thereby decreasing inbreeding.

As pollen settles, nucellar cells collapse, forming a deep depression. Pollen sinks into the depression and germinates. Pollen grains left at the micropyle or in the micropylar canal either do not germinate or germinate late. In Picea and Pinus the integument arms collapse after the pollination drop is withdrawn. In Picea sitchensis (Owens and Blake 1984) this results in a plug which seals the micropyle. In Pinus and Picea a ring of cells inside the micropyle divide and enlarge, constricting and sealing the micropylar canal (Singh 1978, Owens et al. 1981b, Owens and Blake 1984). When a pollen grain germinates, the exine splits and a large pollen tube, formed by the intine, emerges. Several grains may germinate on each nucellus. Sarvas (1962, 1968) suggested that pollen germinating within the nucellar depression has an advantage in effecting fertilization over pollen remaining outside the depression.

In the Cupressaceae, Taxaceae, Taxodiaceae, and Podocarpaceae, which also have a pollination drop (Fig. 5.13), pollen may germinate and elongate within the micropylar canal as in Chamaecyparis (Owens et al. 1980), or after it has reached the nucellus, as in Thuja (Owens and Molder 1980b). Subsequent development is similar to that described above.

In Abies and Cedrus pollen adheres to the funnel-like integument tip (Fig. 5.16). The funnel crimps in and carries the pollen into the short micropylar canal. The nucellus elongates toward the pollen grains and a pollen chamber forms in the nucellar tip, where the pollen may settle (Powell 1970, Singh and Owens 1981b, 1982). In Abies, pollen remains ungerminated within the pollen chamber for several weeks (Owens and Molder 1977d, Singh and Owens 1981b, 1982). Germination then occurs and pollen tubes penetrate the nucellus.

In Larix and Pseudotsuga, pollen is engulfed by the stigmatic tip (Figs. 5.17, 5.18), germinates just inside the sealed micropyle, and then elongates along the micropylar canal. A true pollen tube does not form until the elongated pollen contacts the nucellus. A narrow pollen tube then grows through the nucellus (Allen 1946, 1963, Allen and Owens 1972). Fluid has been incorrectly reported in the micropylar canal of *Larix* (Barner and Christiansen 1960, Villar et al. 1984) and *Pseudotsuga* (Christiansen 1969). This fluid is not present if the micropyle is carefully dissected (Allen and Owens 1972).

Two methods of germination occur in *Tsuga*. In *T. mertensiana*, pollen adheres to the integumentary flaps, germinates, then forms a long pollen tube which grows through the micropyle (Owens and Blake 1983) and along the micropylar canal. The nucellus grows into the micropylar canal and meets the pollen tube which then penetrates the nucellus (Owens and Molder 1975c). In *T. heterophylla*, the spined pollen adheres to the cuticular hairs on the abaxial surface of the bract. Ovuliferous scales overgrow the bracts and after several weeks the pollen grains germinate and form extremely long pollen tubes. The pollen tubes grow over the surface of the bract, through the micropyle and into the nucellus of an ovule on an adjacent ovuliferous scale (Colangeli and Owens 1984). No nucellar attractant has yet been identified. Only the Araucariaceae and *Saxegothaea* of the Podocarpaceae (Doyle 1945) have a pollination mechanism and long pollen tubes similar to *T. heterophylla* (Singh 1978).

Pollen tube development

Pollen tube development in vivo and penetration through the nucellus have been studied very little in conifers. Pollen tube development has been studied in vitro (Ch. 5) and we may assume that physiological and ultrastructural changes are similar in both situations (Singh 1978). Three types of pollen germination have been reported: (1) the exine ruptures irregularly and is cast off in the Cupressaceae, Taxaceae, and some nonsaccate Pinaceae; (2) the intine swells and forms a bubble-like protrusion through a papilla in the exine in the Taxodiaceae; and, (3) the exine splits along a predetermined germinal furrow between the sacci in winged pollen (Singh 1978).

The pollen tube develops from the intine. In germinating *Juniperus* pollen, an external intine is present and ultrastructurally consists of three layers. During germination a new layer, the internal intine, forms next to the plasma membrane by deposition of polysaccharides. The external intine is eventually shed and the internal intine forms the pollen tube (Duhoux 1972a, b). In *Pinus*, the pollen tube arises from an inner pectic-cellulosic layer of the intine (Martens and Waterkeyn 1962). The tube nucleus maintains a constant distance from the tip of the pollen tube and plays an important role in pollen tube growth (Tanaka 1956). Labelled nucleosides fed to germinating pine pollen showed the tube nucleus rapidly incorporated the label, indicating rapid DNA synthesis or turnover for RNA templates and possible enzyme synthesis (Stanley and Young 1962, Young and Stanley 1963). However, Nygaard (1973) concluded that limited RNA synthesis occurred during pollen tube growth. Growth regulators have been isolated from pollen (Ch. 5). These may affect pollen tube growth and ovule development (Sweet 1973). Comparable studies have not been made of possible growth regulators derived from the nucellus.

The pollen tube grows between nucellar cells with some disruption of the latter (Camefort 1978). Willemse and Linskens (1969) demonstrated that germinated and ungerminated pine pollen grains secreted pectinase and cellulase which may dissolve the adhesive layer between cells and cell walls, respectively. Variation occurs in different taxa in rate of pollen germination and pollen tube growth. Germination and pollen tube growth may be completed in two weeks in *Picea engelmannii* (Singh and Owens 1981a), several weeks without interruption of growth in *P. sitchensis* (Owens and Molder 1980a), several weeks with a brief interruption in *Abies amabilis* (Owens and Molder 1977d) or several months with an overwintering dormant stage in *Pinus* (Singh 1978). The function of delayed pollen germination or tube development is uncertain. It could simply be to allow early pollination, with pollen tube and female gametophyte development synchronized later. Willson and Burley (1983) maintain that delayed fertilization may be a female reproductive tactic designed to increase the amount of pollen available, thereby increasing pollen competition and the potential quality of the fertilizing male gamete. Delayed germination does not appear in most conifers because, although pollination mechanisms allow for collection of pollen over time (Ch. 5), in most conifers all pollen grains are taken into the ovule at one time.

Variations in the position of archegonia may alter the method of pollen tube penetration. In the Pinaceae and many of the Cupressaceae (Singh 1978), the unbranched tube grows directly through the nucellus to the archegonia. In *Sequoia*, which has lateral archegonia (Buchholz 1939a, b, Looby and Doyle 1942), tubes grow between the nucellus and the female gametophyte. In some species of *Podocarpus* the tube branches when it reaches the female gametophyte (Konar and Oberoi 1969a, b). These variations demonstrate some of the diversity of materiel available in which to study pollen tube and female gametophyte interactions.

Possible pollen-ovule interactions

In *Larix leptolepis* the stigmatic hairs have been compared to dry-type stigmas of angiosperms (Heslop-Harrison and Shivanna 1977). Esterase activity was demonstrated on the surface of the stigmatic papillae and this could have a function in pollen germination (Villar et al. 1984).

Recognition barriers may exist between pollen or pollen tubes and nucellar or female gametophyte tissue. Such systems have been studied extensively in angiosperms. Pettitt (1977a, b, 1979, 1982) has detected proteins and glycoproteins in the pollen wall, pollen tube wall (intine), and megaspore wall of the primitive gymnosperm *Cycas* and has demonstrated inter-species and inter-tissue precipitation reactions between various components of the ovule tissues. Although there have been no studies of conifers, results from *Cycas* (which has a similar reproductive cycle) suggest that proteins could be important in prezygotic incompatibility during stages of pollen germination, and tube growth through the nucellus, the megaspore wall, and the neck cells. In angiosperms, several experiments have shown that following attachment of the pollen to the stigma, intine proteins are released from the pollen wall as the grain hydrates, and continue to be secreted from the tip of the pollen tube (Pettitt 1982). In angiosperms the proteins located in the pollen tube tip, which encounters the female receptive surface during growth, could be implicated in pollen-stigma recognition (Heslop-Harrison 1976, 1978).

In most conifers the nucellar tip is comparable to the stigma - the site of pollen germination and/or tube penetration. The inner nucellar tissue through which the tubes grow is comparable to the stylar tissue. Several pollen grains commonly reach the nucellar tip but not all germinate, although microscopically they appear living. Their germination could be inhibited by pollen-nucellar interactions. Also, some tubes grow more slowly than others. They are interpreted as being less vigorous, but their slow growth could result from interaction between pollen proteins and nucellar tissue. Little is known about conifer pollen tube wall ultrastructure and proteins except that they are gametophytic in origin in *Juniperus* (Duhoux 1972a, b). In pine pollen, four to six seriologically active substances have been demonstrated (Hagman 1975). Nothing is known about proteins within the nucellar or megaspore cell walls that the pollen tube must penetrate. Some preliminary immunochemical studies have begun in *Larix leptolepis* (Villar et al. 1984) and *Picea abies* pollen (J.M. Pettitt, pers. comm.).

The few immunochemical studies of conifers and the abundant literature dealing with angiosperms indicate this procedure may be a useful avenue of research in conifers. The immunochemical properties of the female gametophyte tissue of *Pinus strobus* were studied in seeds from 6 trees (Eckert and Eckert 1984). Each tree was uniquely identified. This procedure is highly sensitive. Immunofluorescent staining at the light microscope level reveals the presence of specific proteins in the pollen tube wall of *Cycas* (Pettitt 1982). Pollen tube proteins may differ from those of the nucellus and female gametophyte. Interspecific incompatability in *Pinus* (Hagman 1975) and *Picea* (Mikkola 1969) is indicated when pollen tube growth in the nucellus stops early. The chemical activity of enzymes released by the pollen tube could be very specific for complex middle lamellar substances for a species or subgenus (Hagman 1975). These possible barriers to fertilization remain unstudied in conifers. In fact, it is generally accepted, though not proven, that gymnosperms, unlike angiosperms, have limited abilities in prezygotic mate selection (Willson and Burley 1983).

Post-pollination male gametophyte development in hardwoods

In angiosperms pollen germinates on the stigma surface. Variation in the stigma surfaces was briefly mentioned in Chapter 5. The variation found in 1000 species, about 900 genera and 250 families is described and classified into several categories (wet or dry, papillate or non-papillate) by Heslop-Harrison (1977). The stigma type is remarkably consistent at generic levels and even at the family level in some cases. Most hardwood forest trees have dry papillate or non-papillate stigmas. With few exceptions (e.g. Populus) hardwood pollen has one or more germinal pores through which a single pollen tube emerges.

Germination of pollen on the stigma often involves complex interactions between the proteinaceous coating (pellicle) on the stigma surface, the pollen wall, and substances which may be released by both. Pollen rapidly extracts water from the stigma, swells, and releases pollen wall materials. Proteins and glycoproteins derived from the tapetum during pollen development may bind the pollen to the stigmatic surface. Enzymes and enzyme precursors from the intine are then released to degrade the cuticle of the stigmatic papillae and allow the pollen tube to enter (Heslop-Harrison 1975). The time required for these events to happen is short compared to conifers, and may take only minutes (Heslop-Harrison and Heslop-Harrison 1982) or hours (Cresti et al. 1977) depending upon the species. The intimate association between the pollen exine and stigma, and the intine of the pollen tube and style, allow for many sporophytic and gametophytic incompatibility systems to operate (Heslop-Harrison et al. 1975, Heslop-Harrison and Shivanna 1977). Angiosperms generally have more sophisticated prezygotic detection and selection abilities than are thought to occur in gymnosperms (Willson and Burley 1983).

There is commonly a continuity between the glandular stigmatic tissue, the transmitting tissue of the style, and the placental regions of the ovary. Esau (1965) refers to these as stigmatoid tissue because of their cytological and physiological similarity to the stigma. Three types of styles have been distinguished (Vasil and Johri 1964). (1) The style may be open or hollow and the transmitting tissue is represented by a glandular epidermis lining the stylar canal. Pollen tubes grow along this epidermis which nourishes and perhaps chemotropically guides the direction of growth. (2) In most hardwoods, the style is more or less solid and the center is composed of strands of transmitting tissue through which the pollen tubes must pass intercellularly on their way to the ovary. This involves the secretion of pectin digesting enzymes from the tubes to loosen cell walls. (3) Rarely, styles may be half-closed. Styles may be quite long, requiring very long pollen tubes (Foster and Gifford 1974).

The pollen tube forms a complex polysaccharid callose in the innermost lamellae of the intine and this is deposited as callose plugs (Cresti et al. 1977). The function of the plugs is uncertain but is thought to separate active cytoplasm at the pollen tube tip from the older portion of the pollen tube (Stanley 1971). The wall at the tip of the pollen tube is devoid of callose and consists of pectin. The mechanism of pollen tube tip extension may involve changes in calcium ion concentration but the process is not fully understood (Picton and Steer 1982). The pollen tube is strictly gametophytic tissue and it is within the transmitting tissue of the style that gametophytic incompatibilities occur. Tubes are separate so there is no interaction between tubes. Therefore, the rejection reaction is localized to each individual tube. There is no general stylar rejection response.

In such a complex system there are numerous stages at which normal development could be slowed or stopped. In Populus, enzymes are released from the pollen wall which are involved in emergence and nutrition of pollen tubes as well as stigma penetration. Other proteins released are considered to be recognition substances. This is the basis for the recognition or mentor pollen pollination technique (Stettler 1968) in which dead compatible pollen is mixed with fresh incompatible pollen. The dead pollen provides recognition substances essential for stigma penetration and growth of the incompatible pollen tubes (Knox et al. 1972b). Stigma type and pollen type have also been related to the incompatibility system (Heslop-Harrison and Shivanna 1977). Although very little work has been done for hardwood forest trees, similar systems to those already found in herbaceous plants probably operate.

Incompatability in forest trees

The term "incompatibility" is often misused. Following Hagman's (1975) definitions, incompatibility is the situation in which functional male and female gametophytes cannot unite because fertilization is blocked at some point. Incompatibility can thus be intraspecific or interspecific. It involves prezygotic detection and selection abilities which are highly developed in angiosperms (Willson and Burley 1983). Inviability is where development of the new individual is arrested at some point after division of the zygote. This postzygotic detection and selection is well developed in gymnosperms (Willson and Burley 1983).

In conifers, self-incompatibility has not been reported, rather, pollen tube growth and fertilization are thought to proceed normally but embryos abort at an early stage of development. This has been observed in Pinus (Hagman and Mikkola 1963, Forshell 1974), Pseudotsuga (Orr-Ewing 1957, 1965), and Picea (Andersson 1965, Mergen et al. 1965). Numerous other reports show similar low seedsets from self pollination but have looked only at mature seed rather than the stages at which development stops. There is general agreement that low seedset resulting from self pollination is caused by embryo abortion (Hagman 1975).

Interspecific incompatibility is common in conifers and results from inhibition of pollen germination or pollen tube growth (McWilliam 1959a, Li 1964, Mikkola 1969, Hagman 1975, Nakai et al. 1976, Kormutak 1984). This has been extensively studied in Pinus where it is common in the subgenus Diploxylon but rare or absent in the subgenus Haploxylon. Again, few studies have followed development. Instead they have relied on indirect evidence of cone abscission, due to the inability of pollen to induce ovule development (Hagman 1975). In interspecific crosses of Pinus, Kormutak (1984) found that if pollen germination on the nucellus was inhibited, the conelets aborted in the first growing season; however, if pollen germinated and the pollen tube penetrated the first layers of the nucellus but fertilization did not occur, the ovule aborted during the second season resulting in empty seed. Developmental studies to determine the stage at which interspecific incompatibility occurs are absent for other conifers.

Intraspecific incompatibility, if present in conifers, is less developed than in angiosperms. Many forest geneticists have made crosses in conifers in which seedset is poor. The stage at which the system fails has not been determined but could result from recognition barriers. Some indirect evidence suggests this is possible. Different sugars have a species-specific effect on pollen germination and tube growth in pines (Chira and Berta 1965), and irradiation of pollen changed the sugar composition and allowed hybridization of P. nigra and P. sylvestris (Vidakovic and Jurkovic-Bevilacqua 1971). Another possibility is that a high degree of disparity between pollen and nucellus might stimulate the synthesis by the nucellus of phytotoxic substances such as phenols which may inhibit pectic enzymes (Hagman 1975).

Incompatibility in hardwood species (Hagman 1975) has been infrequently studied; however, the literature on herbaceous angiosperms is extensive. It is reasonable to assume that hardwood forest trees would have patterns similar to other angiosperms. It is beyond the scope of this review to cover the latter but here are several recent papers and reviews (Hagman 1975, Heslop-Harrison 1975, Heslop-Harrison et al. 1975, Heslop-Harrison and Shivanna 1977, Nettancourt 1977, Kormutak 1984).

Self incompatibility in hardwoods occurs in the style and is expressed through retarded pollen tube growth. This has been described in Alnus (Hagman 1970), Betula (Hagman 1971), and Quercus (Pjatnitsky 1947). Details are not known for Fagus and Castanea but, because of similarities with Betula and Alnus, Hagman (1975) suggests that the former should have a similar system. Interspecific incompatibility has been studied in Populus with the goal of obtaining hybrids between incompatible species (Stettler et al. 1980). Pollen-stigma interactions examined using the scanning electron microscope identified considerable variation especially in pollen size, hydration, and tube length. Some of these variations were significant in determining the fate of matings. These observations do not support the hypothesis by Knox et al. (1972b) that a protein released by the exine sets the stage for germination and pollen tube growth in compatible crosses. Stettler et al. (1980) found no differences in the early stages between compatible and incompatible pollen-stigma interactions and attached more significance to post-germination stages. In contrast, in Ulmus, interspecific incompatibility occurs at the stigma surface (Ager and Guries 1982). Solvent treatment of pollen and stigmas, mentor pollen, or extracts from compatible pollen, have been used to increase hybridization of incompatible poplars (Stettler and Bawa 1971, Whitecross and Willing 1975, Willing and Pryor 1976, Stettler et al. 1980).

Seed efficiency in hardwoods is often low (Schopmeyer 1974, Willson and Burley 1983). Future studies of the poorly understood pollen-stigma and pollen tube-style interactions in commercial hardwoods may provide explantations for low seed efficiencies. Some impediments may be overcome by the manipulations which have been used to increase crossability in hardwoods.

Female gametophyte development in conifers

Each ovule contains a megaspore mother cell during winter dormancy. Meiosis commonly occurs about the time of pollination. Female gametophyte development occurs during the next one to two months except in some genera (eg. Pinus) where this takes over one year. Meiosis produces four megaspores; three degenerate and the functional megaspore undergoes several weeks of free nuclear division during which several hundred free nuclei form. Cell walls develop between all nuclei, forming a multicellular female gametophyte. Usually, several cells at the micropylar end of the female gametophyte function as archegonial initials. Each initial enlarges and divides unequally, producing a small primary neck cell and a large central cell. The former divides to form one or more tiers of neck cells. The central cell enlarges and divides unequally to form a small ventral canal cell and large egg cell (Fig. 6.1). The egg cell becomes filled with large and small inclusions containing stored lipid and protein (Singh 1978). Female gametophyte (prothallial) cells often incorrectly called "endosperm" also become filled with stored food. However, endosperm has a different origin and genetic makeup and often a different function than the female gametophyte.

The egg cell is enclosed by an archegonial jacket. The archegonial jacket, neck cells, ventral canal cell, and egg constitute an archegonium (Fig. 6.1). The number of archegonia varies with species. Willson and Burley (1983) give an extensive list of species and variation in archegonial numbers. Variation within species often results from differences in size and shape of the female gametophyte (Owens and Molder 1984a). Singh (1978) described some of the variation found in female gametophyte development, and references therein provide descriptions of many species. Most conifers have several archegonia at the micropylar end, each separated from the others by sterile tissue. In the Pinaceae, archegonia range from one to ten but most commonly number three to five (Willson and Burley 1983). In the Cupressaceae and Taxdiaceae several archegonia have a common jacket forming an archegonial complex. The number of archegonia per archegonial complex may vary from 5 to 100 (Singh 1978, Willson and Burley 1983). In Sequoia several archegonial complexes occur laterally (Buchholz 1939a, b, Looby and Doyle 1942).

Female gametophytes may abort at various stages of development. Abortion commonly occurs at or shortly after meiosis in many Pinaceae and Cupressaceae (Owens and Molder 1984a-d). Ovule development becomes arrested and a small flattened empty "seed" results. The causes of this are uncertain but low temperature at pollination has been suggested (Owens and Molder 1980b). In most conifers, pollen is not essential for continued female gametophyte development, whereas in Pinus, pollen must be present in the nucellus for ovules to develop (McWilliam 1959a, Sarvas 1962, Sweet 1973, Plym Forshell 1974, Owens et al. 1981b). In Picea the presence of

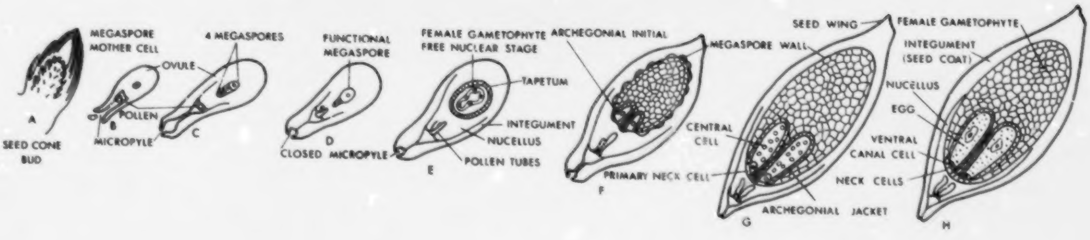


Figure 6.1 Ovule and female gametophyte development in the Pinaceae. A. The dormant seed-cone bud. B-H. Ovule development. B-E. Predormancy development. F-H. Postdormancy development (from Owens and Molder 1984b).

pollen may also be essential for ovule development (Mikkola 1969, Kossuth and Fechner 1973, Fechner 1979, Owens and Blake 1984). Originally it was proposed that auxin from the pollen stimulated increased auxin production by the ovule, which in turn attracted increased metabolites thus reducing ovule abortion (Sweet and Lewis 1969). It was subsequently shown that pollination did not cause increased movement of ^{14}C to the ovule and exogenous application of auxin had no clear-cut effect (Sweet 1973). Sweet (1973) has postulated that the pollen tube may release serological substances which stimulate ovule development. Most developmental studies emphasize normal formation and do not look for frequency or timing of ovule abortion; therefore, it may be more important in seed production than the literature indicates.

Female gametophyte development in hardwoods

The ovary may consist of several carpels, each of which may contain several ovules. Each ovule contains a megaspore mother cell which undergoes meiosis to form four megaspores (Fig. 6.2). From this stage there is a surprising diversity in female gametophyte (embryo sac) development. Variation occurs in: (1) the number of megaspores or megaspore nuclei that participate; (2) the number of nuclear divisions; (3) the occurrence of nuclear fusions; and, (4) the number, arrangement, and chromosome number of the cells and free nuclei present (Foster and Gifford 1974). Reviews of classifications recognize at least 11 categories of embryo sac development (Johri 1963, Foster and Gifford 1974). Davis (1966) gives the type of female gametophyte development found in many angiosperm families.

The category of embryo sac development in hardwood forest trees appears to be the monosporic seven-celled (Polygonum) type (Fig. 6.2) (Davis 1966). This type is the most common type in angiosperms. Following meiosis, three of the four megaspores degenerate. The functional megaspore divides to form a binucleate structure with one nucleus at each pole of the embryo sac. Each nucleus divides. Then these daughter nuclei divide forming an 8-nucleate embryo sac with four nuclei at each pole. Three of the nuclei at the micropylar pole become differentiated; one as the egg cell and two as synergids or the egg apparatus. At the opposite end three of the four nuclei become differentiated as antipodal cells. The two remaining nuclei (polar nuclei) migrate from the opposite poles and form

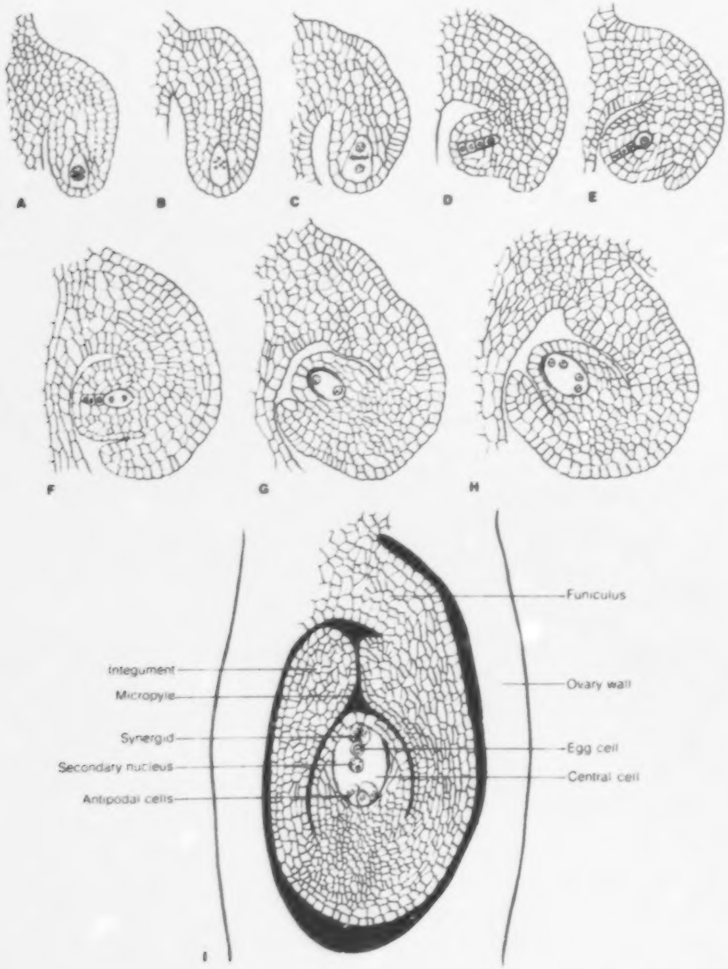


Figure 6.2 Ovule and the Polygonum type of embryo sac development. A. Ovule containing a megaspore mother cell. B, C. Meiosis of megaspore mother cell. D. Four megaspores. E, F. Three degenerating and inner functional megaspores. G, H. Two and four free nucleate states. I. Mature embryo sac (from Foster and Gifford 1974).

the binucleate central cell. The polar nuclei may remain separate or fuse before fertilization to form the diploid secondary nucleus (Fig. 6.2) (Johri 1963, Foster and Gifford 1974). Multiple embryo sacs are rare but Alnus rugosa is an exception. It forms one to four embryo sacs per ovule apomictically (Davis 1966). The occurrence of multi-ovulate carpels is common and parellels the multiarchegoniate condition in conifers (Willson and Burley 1983) but some ovules abort during development. Early abortion occurs in Quercus (4 of 5 abort) (Mogensen 1975) and Betula (3 of 4 abort) (Clausen 1973a). The cause or function of ovule abortion is unknown. It occurs regardless of levels of pollen received or nutrition. Early abortion of ovules may also occur in other hardwoods and affect seedset, but this has not been studied.

In most hardwood forest trees the phenology of ovule development is unknown; therefore, the stage of female gametophyte development cannot be related to climatic conditions or time of pollination. Because the time between pollination and fertilization is short in most angiosperms, it is safe to assume that female gametophyte development is usually fairly advanced at pollination (Conrad 1900, Nagaraj 1952, Hjelmqvist 1953, Germain et al. 1973, Mogensen 1975).

Fertilization

Discussions of fertilization may include all stages, from pollen structure through gamete fusion (syngamy) (Linskens 1964, 1974) to more restricted usage including only the release of male gametes and fusion with the female gamete (Singh 1978). It represents the transition from the haploid to the diploid phase of development (Linskens 1974). In this review, fertilization will include male gamete formation and release and their fusion with female gametes.

Fertilization is essential in seed production of all commercially important hardwood and conifer species. The term "parthenocarpy" is often misused in forestry literature and should be restricted to refer to the formation of a fruit without fertilization of ovules. The term "parthenogenesis" should refer only to the formation of an embryo without fertilization (Nitsch 1963). This usage will be adhered to in this review. Others have used parthenocarpy to designate fruit formed without pollination, without seeds, or with empty seeds (see Nitsch 1963).

Conifers

In conifers fertilization does not appear to be a stage where obvious impediments occur. If pollen tubes reach the archegonium, fertilization generally follows. However, this generalization is based upon descriptive studies in which chemical or ultrastructural impediments would not be detected. The normal sequence is for two male gametes to form by division of the body cell, usually during the latter stages of pollen tube growth. Male gametes may be: two equal sized cells in the Cupressaceae, Taxodiaceae, and Araucariaceae; two unequal cells in some Taxaceae and Podocarpaceae; or two equal sized nuclei enclosed within the body cell cytoplasm, as in the Pinaceae and Cephalotaxaceae (Singh 1978).

Pollen tubes usually penetrate the magaspore wall where it is often thinner over the archegonia. Nothing is known of this process but it must involve dissolution of the megaspore wall and/or the pollen tube wall. The megaspore wall consists of a suberized outer (exosporum) and double cellulose-pectinaceous inner (endosporum) layer comparable to the exine and intine, respectively, of pollen. The interpretation of this wall has been questioned (Singh and Johri 1972). The physical or chemical constituents of the wall could be an important sporophytic (exine) or gametophytic (intine) barrier to fertilization and needs further investigation.

In most conifers the neck cells degenerate and the pollen tube penetrates the ventral canal cell and releases both male gametes inside an archegonium (Singh 1978). Some studies show pollen tubes separating intact neck cells and then penetrating the ventral canal cell (Owens and Molder 1977c). In conifers having archegonial complexes several pollen tubes may extend to each complex. In Thuja plicata two male cells form just above the intact neck cells. The latter then degenerate and male cells enter the egg cell (Owens and Molder 1980b). In conifers with archegonial complexes it may be possible for the two male cells from one pollen tube to fertilize different egg cells. Other variations may also occur (Singh 1978).

A receptive vacuole forms where the pollen tube enters into the egg cytoplasm. In addition to male gametes, other cells and nuclei from the pollen tube may enter the egg. These supernumerary cells and nuclei (Allen and Owens 1972) usually degenerate. One male gamete migrates to the center of the egg cell, the other remains near the receptive vacuole and is non-functional (Fig. 7.1). Double fertilization does not occur. In some gymnosperms the non-functional male gamete may fuse with the ventral canal cell and then divide irregularly, but few cells result (Singh 1978).

Of some importance to seed production and, especially, genetics is the cytoplasmic contribution of gametes to the zygote. Ultrastructural studies show that a perinuclear zone around the egg nucleus contains many mitochondria. Fusion of the male gamete and egg nucleus is accompanied by the formation of a dense neocytoplasm (Camefort 1969) around the zygote nucleus and first cells of the proembryo. In Pinus, early reports claimed the male cytoplasm did not enter the egg (Camefort 1969), but Willemse (1974) showed that plastids do enter with the male gamete. In Larix, the male gamete carries a small amount of cytoplasm with plastids and mitochondria into the egg (Camefort 1968, Chesnoy and Thomas 1971). Any female plastids in the egg cell of Pinus and Larix appear to degenerate. Thus mitochondria are contributed by both male and female parents but plastids come only from the male parent. A similar situation appears to exist in Pseudotsuga (Thomas and Chesnoy 1969). In Biota, representing the Cupressaceae, the male gamete contributes considerable cytoplasm, mitochondria, plastids, and cytoplasmic RNA to the egg. However, few maternal mitochondria and no maternal plastids are included in the neocytoplasm (Chesnoy 1969). The male contribution is similar in Chamaecyparis (Chesnoy 1973). Genetic proof for paternal transmission of plastids has been shown in Cryptomeria of the Taxodiaceae (Ohba et al. 1971).

Hardwoods

Fertilization in angiosperms is more variable than in conifers and has been the subject of reviews (Steffen 1963, Kapil and Bhatnagar 1975), symposia (Linskens 1964, 1974), and texts (Maheshwari 1950). Variation occurs in the method of pollen tube entrance into the ovule, fusion of male and female cells, and in endosperm development. The processes are similar within each plant family and in most commercial north temperate hardwood forest trees, but there are no detailed ultrastructural studies of fertilization for hardwood trees.

After reaching the ovary, the pollen tube may enter an ovule by several routes (Kapil and Bhatnagar 1975). The most common route in herbaceous species is called porogamy (Foster and Gifford 1974). Here the pollen tube grows from the transmitting tissue into the locular space around the ovules, through the micropyle, and then through the nucellar tissue to the egg apparatus. This has not been reported in common hardwood genera, although many species have yet to be described. Another type, found in Betulaceae, Juglandaceae and Ulmaceae (Davis 1966) is the chalazogamous route. Here, the tip of the pollen tube penetrates the chalazal end of the ovule (opposite the micropyle) then grows along the surface of the embryo sac to the egg apparatus. In porogamous and chalazogamous routes the pollen tube finally penetrates the micropylar end of the embryo sac (Foster and Gifford 1974).

Entrance into the egg apparatus appears to be variable (Kapil and Bhatnagar 1975). The pollen tube may pass between a synergid and the egg, between a synergid and the embryo sac wall, or into a synergid. The two male gametes are released into a synergid. By an unknown mechanism, one gamete enters the egg, the other enters the central cell and the synergid degenerates (Steffen 1963, Foster and Gifford 1974).

Double fertilization occurs in angiosperms: one male gamete fuses with the egg to form the zygote, and the second gamete fuses with one or more polar nuclei to form the primary endosperm nucleus. The method of gamete movement is unknown (Steffen 1963, Foster and Gifford 1974, Kapil and Bhatnagar 1975). In the families which include most north temperate forest trees, the polar nuclei fuse to form a diploid (2n) fusion nucleus before fertilization (Davis 1966). Subsequent fusion with the male gamete produces a triploid (3n) primary endosperm nucleus.

Endosperm is the tissue formed from the primary endosperm nucleus during seed development. The endosperm provides food materials essential for growth of the embryo and often the young seedling. Therefore, it is functionally analagous to the haploid female gametophyte of gymnosperms, but different in origin and genetic makeup. Considerable variation exists in the development of endosperm (Foster and Gifford 1974). It may consist entirely of a liquid mass of multinucleate cytoplasm, or the free nuclear phase may be followed by centripetal cell wall formation resulting in cellular tissue. The latter type appears to occur in most hardwood forest trees, except Fraxinus (Davis 1966). In Fraxinus the endosperm is cellular from the start; cell wall formation follows division of the primary endosperm nucleus. In the Fagaceae, including Fagus and Quercus, the endosperm, after a free-nuclear phase becomes cellular, except at the chalazal end where a multinucleate mass of cytoplasm remains (Davis 1966). The histological structure and the types of food reserves in endosperm vary widely (Maheshwari 1950). The function of endosperm and female gametophyte in embryo and seed development are discussed in Chapter 7.

Summary, and recommendations for future research

Gametophyte development and fertilization involve many complex stages, each essential in seed development. However, in most forest trees the effect of a malfunction or developmental anomaly at any stage is uncertain.

In angiosperms the pre-fertilization stages are very important in seed production. Barriers to fertilization have been identified in many species, and studied extensively in herbaceous species. Similar studies have begun in a few hardwood forest trees. The use of ultrastructural and immunochemical techniques have clarified pollen-stigma interactions and incompatibility responses in angiosperms, but relatively few studies have utilized hardwood species. More research on hardwoods is needed.

Conifers are generally thought not to have pre-fertilization recognition mechanisms. However, developmental studies of pollen-nucellus interactions suggest that this generalization may be premature. Knowledge of pollen tube development and growth through the nucellus to the egg is much less extensive in conifers than in angiosperms and research needs to be done in this area. Immunochemical reactions between gametophytic and sporophytic tissues have been demonstrated in primitive gymnosperms using established techniques. Research coupling ultrastructural and immunochemical techniques applied to conifers could determine some causes of poor seed crops. The production of possible pollen tube attractants by the nucellus and female gametophyte should also be investigated.

The frequency of early ovule abortion is significant in most conifers but uncertain in many hardwoods. Environmental and physiological factors causing early ovule abortion should be determined.

Fertilization is not well understood in conifers or in commercially important hardwoods. Until it is better understood, we will not know if there are problems to be overcome for increased seed yield.

There is some evidence that the number of seeds per cone or fruit varies with each parent tree regardless of self/cross ratios (Willson and Burley 1983). That is, some trees are more successful mothers than other trees. The reasons for this are unknown but may be related to resource competition or allocation in the parent tree, incompatibilities, or developmental problems. These and other potential causes must be investigated for each commercially important tree species.

CHAPTER 7

EMBRYO AND SEED DEVELOPMENT

Introduction

Embryogeny is the development of the embryo. The earliest stages of the embryo of gymnosperms and angiosperms are designated as the proembryo. This stage ends when a filamentous row of cells (usually about 16) form. A portion of the cells develop into the embryo. The embryo of gymnosperms develops within the haploid female gametophyte and that of angiosperms within the endosperm, which has variable ploidy. Beyond a few generalizations which apply to both gymnosperms and angiosperms, details of embryogeny differ markedly between the two groups. Considerable variation also exists within each group; consequently, embryogeny of conifers and hardwood forest trees will be discussed separately. Although embryo formation is an important, often fragile process, few studies have attempted to identify aspects of embryogeny that causes poor seed production. Most studies of embryogeny are descriptive and morphological. General discussions of embryogeny are given by Johansen (1950), Wardlaw (1955), and Foster and Gifford (1974). Discussions of gymnosperm embryogeny are given by Schnarf (1933), Chamberlain (1935), Roy Chowdhury (1962), and Singh (1978), and angiosperm embryogeny is discussed by Maheshwari (1950, 1963).

Embryogeny of Conifers

Conifer embryogeny has been studied in two ways: one descriptive and developmental, and of academic interest in botany (Johansen 1950); and the other for seed testing in forestry (Simak and Gustafsson 1953a, b, 1954). There have been several reviews of conifer embryogeny (Buchholz 1926, 1929, Doyle 1957, Roy Chowdhury 1962, Konar and Oberoi 1969a, Singh 1978), but few studies have dealt with embryological details required for seed quality and genetic analysis (Sarvas 1962, Dogra 1967). Dogra's (1967) approach was unique; he used embryogeny to determine seed quality and sterility in natural and controlled breeding populations. He showed that embryo mortality increases the number of empty seeds. Embryological disturbances are found in all conifers and may occur any time during embryo development. Disturbances may be caused by climatic or genetic factors, but it is difficult to distinguish between these. Generally, embryo mortality is higher in early- than in late-embryo stages (Dogra 1967).

The literature on conifer embryogeny is extensive and species from all families and most genera have been described (Roy Chowdhury 1962). The Pinaceae have been studied most extensively. Proembryogeny in the Pinaceae was recently reviewed by Mehra and Dogra (1975) and this, and later, stages were reviewed by Dogra (1967) and Singh (1978). Dogra (1967) thoroughly reviewed embryogeny variation within the Taxodiaceae. Chesnoy's (1977) study of Biota orientalis gives an extensive literature review of the Cupressaceae. Few species in the Podocarpaceae have been studied and the most recent review is by Buchholz (1941). The embryogeny of most genera within the Taxaceae has been described but there is no review of that literature. However, a recent study of Pseudotaxus includes most references to embryogeny within the family (Chen and Wang 1978). Cephalotaxus, the only genus within the Cephalotaxaceae, has been studied and its relationship to other conifers evaluated (Singh 1961, 1964). The Araucariaceae of the southern hemisphere has been studied very little (Singh 1978).

Proembryo development begins with the division of the zygote nucleus. In the Pinaceae, two daughter nuclei form and divide, producing four free nuclei enclosed by dense neocytoplasm in the center of the archegonium. The four free nuclei and the neocytoplasm migrate to the chalazal end of the archegonium and form a single tier of nuclei. Nuclear division and cell wall formation follow, forming an 8-celled then a 12- or 16-celled proembryo (Fig. 7.1). Proembryo cells may vary in number and arrangement according to family and genus within a family (Roy Chowdhury 1962). The terminology associated with these variations is extensive (Roy Chowdhury 1962, Dogra 1967, Singh 1978). Generally, irregularities which occur during proembryo development have not been related to seedset.

Since most conifers are multiarchegoniate, more than one egg may be fertilized. Multifertilizations usually occur about the same time and resultant proembryos develop at a similar rate.

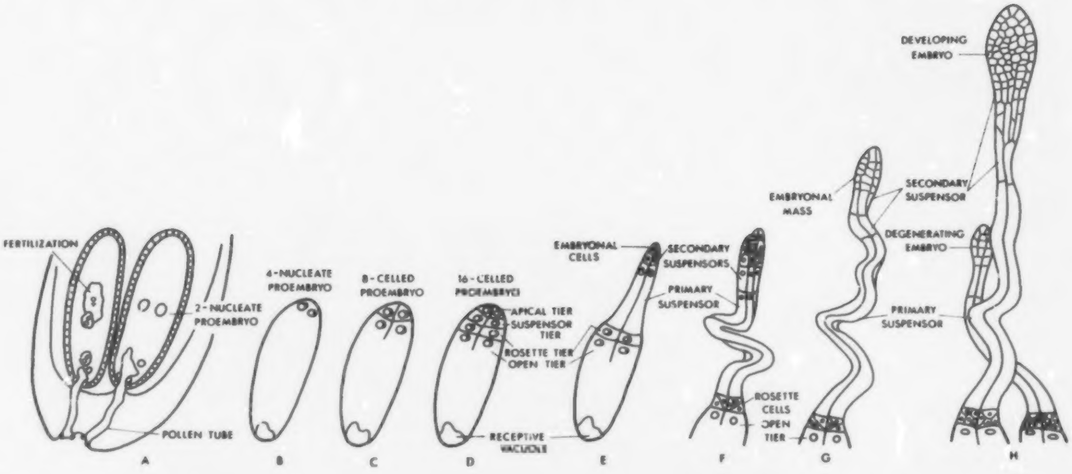


Figure 7.1 Fertilization and early embryo development in Picea (from Owens and Molder 1984c).

Fertilization of one egg in a female gametophyte does not appear to prevent subsequent fertilization of other eggs; however, unfertilized eggs rapidly degenerate as adjacent proembryos develop. The fertilization of more than one egg per female gametophyte, simple polyembryony (SPE), is very common but not universal in conifers (see Appendix in Willson and Burley 1983). The resulting embryos have different genotypes. Rarely does more than one proembryo develop into an embryo. The mechanism by which the successful embryo inhibits the others, usually resulting in their abortion, is unknown. Willson and Burley (1983) discuss SPE in terms of mate choice by both males and females. The tendency towards increased number of archegonia per ovule helps reduce the occurrence of empty seed (Sarvas 1962). Doyle (1954) demonstrated the tendency for archegonial number to increase from primitive to derived members of the Podocarpaceae. Similar studies have not been made for other families. Archegonial number may vary within populations and could be a trait to select for in seed orchards.

The proembryo stage ends when the suspensor cells (Fig. 7.2) within the proembryo elongate and force the apical (embryonal) tier into the female gametophyte. Cells of the apical tier divide and may form a single embryo or they may separate into four filaments of cells, each of which may develop into a separate embryo. This is called cleavage polyembryony (CPE) and the embryos are genetically identical. Buchholz (1929) arugued that a single large proembryo should be able to prevail over a small cleavage embryo, but Doyle and Brennan (1971) noted that cleavage embryos grow faster. Willson and Burley (1983)

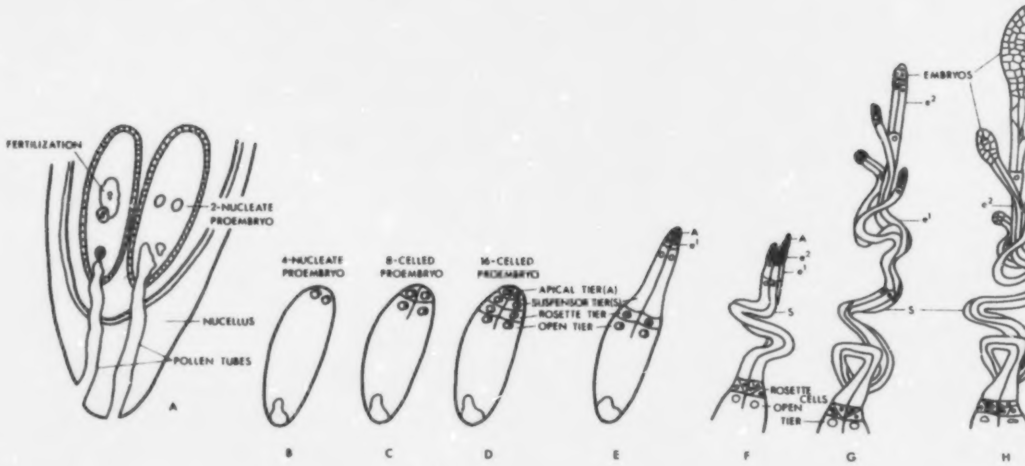


Figure 7.2 Fertilization and cleavage polyembryony in *Pinus* (from Owens and Molder 1984b).

propose that the CPE embryos may assimilate nutrients faster than a single large embryo. This may intensify competition between embryos of different genotypes within the same ovule. Not all conifers undergo CPE, and others only rarely (see Appendix in Willson and Burley 1983). Often, in those lacking CPE, all cells of the proembryo do not contribute equally to embryo development. Some files of cells are more vigorous and overgrow the others but do not separate them. This has been called delayed cleavage polyembryony and has been observed in *Pseudotsuga* (Allen and Owens 1972), *Larix* (Schopf 1943, Owens and Molder 1979c), and *Picea* (Singh and Owens 1981a). Its significance is uncertain. It may only be an evolutionary intermediate from CPE to SPE (Buchholz 1926, 1950) or from SPE to CPE (Thomson 1945 and Roy Chowdhury 1962). Other categories of polyembryony are discussed by Dogra (1967).

Conifer embryo development may continue normally, may be inhibited but resume when conditions are favorable, or the embryos may degenerate. In *Pinus* (Hagman and Mikkola 1963), *Pseudotsuga* (Orr-Ewing 1954, 1957), and *Picea* (Mergen et al. 1965), degeneration has been traced to self pollination and may be a result of physiological incompatability between early embryos and the female gemetophyte tissue. Observations on other conifers (eg. *Abies*), however, do not show that self pollination leads to embryo degeneration (Sorensen 1982). Defective or undeveloped seeds due to selfing were seldom found in *Pinus strobus* (Kriebel 1966). The embryology of self pollinated conifers has not been fully investigated. It has been stated that intraspecific prezygotic self incompatability has not been reported for conifers (Hagman 1975) but that selection against selfing occurs by low self embryo viability (Sorensen 1982).

In interspecific crosses of *Picea* and *Pinus*, the incompatability barrier is commonly prezygotic (Dogra 1967, Hagman 1975). Pollen tube development is usually inhibited (Hagman 1975) but if fertilization does occur, embryo degeneration may follow (Buchholz 1944). Proembryo mortality is one of the causes of poor seedset in interspecific crosses of some pines (Wright 1959, Hagman and Mikkola 1963).

Dogra (1967) mentions several proembryo irregularities which others (Wardlaw

1955, Owens and Molder 1975b) considered as natural variations in cell number and arrangement, determined in part by changes in shape and size of female gametophytes and archegonia. These variations may not adversely affect embryo or seed development. However, some authors view any variation as an irregularity which may result in an abnormal embryo and increased embryo mortality. Dogra (1967) observed that the occurrence of any one abnormality may not be common, but all abnormalities totalled 2 to 15 per cent of the embryos for a species. He concluded that early embryo mortality played a more significant role in conifer seed sterility than is presently thought. Looby and Doyle (1937) also commented that the frequency of abnormal embryos was probably more widespread than can be judged by published reports. Unfortunately, large samples and numerical data are generally lacking for conifer embryological studies.

Embryo degeneration may occur at several stages and result in normal appearing seeds with well formed female gametophytes and embryo cavities but with degenerated embryos (Müller-Olsen and Simak 1954). On this basis, Dogra (1967) described four classes of seeds. In Class I seeds the ovules are pollinated and the eggs fertilized, but something causes the young embryos to abort. Class II seeds may contain well developed female gametophytes and one or more small embryos not longer than half of the embryo cavity. This class may be further subdivided (Simak and Gustafsson 1959, Simak and Kamra 1963, Simak 1966). Class III seeds contain one or more embryos, the longest of which measures between half and three quarters of the embryo cavity. Class IV seeds have one fully developed embryo completely or nearly completely filling the embryo cavity. He separated seeds of *Pinus sylvestris* and *Picea abies* into these classes and determined possible causes of degeneration in each class. This approach could be applied to other conifers in order to understand the underlying causes of good or poor seed-set. However, Dogra's system may not adapt well to all conifers since, unlike many other conifers *Pinus* (Sarvas 1962), and to a lesser extent *Picea* (Sarvas 1968, Mikkola 1969) require the presence of pollen for normal prefertilization ovule development.

Berlyn (1962) generalized that in *Pinus*, large seeded species had a more prolonged selection period than small seeded species. However, this was not true within a species and Buchholz (1946) found that, in a given tree, the smallest seeds had the longest period of embryonic selection. If embryonic selection occurs very late in development, polyembryonic seeds may result (Johnstone 1940, Berlyn 1962). The occurrence of polyembryonic conifer seeds has frequently been reported (Johnstone 1940).

Deficient ovules

Deficient ovules may result from ovule abortion or a lack of ovule development. Ovule abortion has been most extensively studied in *Pinus* (Sweet 1973) where pollen is necessary for normal ovule and female gametophyte development. Similar observations have been made for *Picea* (Sarvas 1968, Mikkola 1969, Owens and Blake 1984). However, in *Picea* a small number of unpollinated ovules develop and pollination with dead pollen or pollen from other species may promote ovule development. Sweet and Lewis (1969) suggested that diffusible auxin from pollen in the nucellus may stimulate the ovule to produce hormones that promote its further development. The absence of pollen in most other conifer genera does not stop female gametophyte development and normal appearing seeds will form. However, without fertilization no embryos are present and the female gametophyte degenerates leaving a normal sized, but empty, seed (Orr-Ewing 1957, Owens and Molder 1975b, c, 1977d, 1979a, Singh and Owens 1981b, 1982).

All conifer cones have ovuliferous scales at the base, and to a lesser extent at the tip, which bear either no ovules or rudimentary ovules. Rudimentary ovules either do not fully develop or develop slowly and are not pollinated (Owens et al. 1981a, b). This is most marked in *Pinus*. Sarvas (1962) demonstrated that 78 per cent of the scales in *P. sylvestris* were deficient or sterile and 92 per cent of these were at the base of the cone. Only 60 per cent of *P. radiata* (Sweet 1973) and 25 per cent of *P. contorta* (Owens et al. 1982) scales bore ovules capable of forming seeds. This severely limits seed potential of cones but is inherent in the species and probably cannot be altered. This trait should be accurately determined for each species so that potential seed production or seed efficiency (Bramlett et al. 1978) is not overestimated.

Deficient ovules also occur in the fertile regions of cones, but this has not been carefully studied with regard to cause or frequency within cones, trees, or species. Lyons (1956) found that 50 to 60 per cent of ovules in the fertile region of *P. resinosa* cones produced no seed and attributed this to ovule abortion in the first year or failure to produce archegonia in the second year. In *Pseudotsuga* in New Zealand, deficient ovules ranged from 13 to 52 per cent, which was influenced by geography and climate (Sweet and Bollmann 1972). In *Picea abies* about 7 per cent of ovules were deficient (Sarvas 1968). In deficient ovules resulting from ovule abortion, seed wings may develop but seeds are commonly small and flat. Also, since seed wings in the Pinaceae develop from the ovuliferous scale (Owens and Smith 1965) it is possible to have wings develop without an attached ovule. Failure to recognize the possible deficiencies in the fertile regions of cones can result in miscalculations of ultimate seed yield (Lyons 1956).

The most common cause of late ovule abortion in some genera is the lack of pollen; however, other factors may also have an effect. Lyons (1956) and Burdon and Low (1973) suggest that competition for nutrients may be a factor leading to abortion of pollinated ovules. Dickman and Kozlowski (1968, 1969a, b, 1970) have demonstrated seasonal growth patterns in *Pinus resinosa* seed cones and changes in macro- and micronutrients in developing seeds. Developing seed cones are strong metabolic sinks competing for finite amounts of nutrients. Therefore, any general increase in tree vigor may increase cone and seed production. Release by removal of adjacent trees, which generally increases nutrients available to the remaining trees, increased cone production and the proportion of sound seed (Allen and Trousdell 1961).

Some irregularities in ovule and seed development may be environmentally caused. Brown (1973) suggests factors such as site and crown position as well as inherent traits of individual trees may influence seed development. Damage to the female gametophyte and embryos of *P. sylvestris* and *Picea abies* has been caused by subarctic climate (Simak and Gustafsson 1954, Sarvas 1962) and by drought for *Cedrus deodara* and *Abies pindrow* (Dogra 1967). In *Thuja plicata*, slightly lower than normal temperatures at pollination were related to a high incidence of ovule abortion (Owens and Molder 1980b). Dogra (1967) stated that climatic factors generally have less effect on proembryogeny than on late embryogeny. The degree of damage may vary with the stage of development and resistance of the species, provenance, or tree.

There have been several impediments to studies of endogenous and environmental effects on ovule development. Few studies have been made on the physiological changes occurring in gametophytes and embryos during development (Takao 1960). Until recently it was not possible to test endogenous and environmental factors on reproductive trees under controlled conditions. However, advanced biochemical, histochemical, and ultrastructural techniques, combined with cone induction on small trees (Ch. 4) may make these studies possible.

Cone abortion and loss

Cones, like seeds, may abort or otherwise be lost before seeds are mature. The causes are many, are often poorly understood, and may vary with the species. The most susceptible stage is at or about the time of pollination. This is usually assumed to result from low temperatures (Hard 1963, Hutchinson and Bramlett 1964, Krugman 1966) but has not been carefully monitored under controlled conditions. Death of the conelet commonly begins in the cone axis (White and Knopp 1978) and ovules, followed by wilting and browning of the bracts (J.N. Owens, pers. observation). To determine temperature and humidity effects on conelets, studies should be done on small trees maintained in controlled environments.

Few studies deal with pre-pollination loss of conelets, except those dealing with potential cone-bud abortion or latency (see Ch. 3). There is some evidence in *Pinus* that 3 to 5 per cent of conelets abort during the 3 months prior to pollination (Seet 1973). Sweet and Bollman (1970) attributed some early conelet loss (drop) to competition between strobili and vegetative apices in *P. radiata*.

Most studies of conelet loss are on *Pinus*, where post-pollination conelet abortion is common. This can usually be traced back to inadequate pollination. Sarvas (1962) postulated that 80 per cent of naturally occurring conelet drop in P. sylvestris resulted from inadequate pollination and the remaining 20 per cent resulted from some type of damage to the conelet. He estimated that when more than 20 per cent of potentially fertile ovules aborted (due to lack of pollen) the conelets die during the first year of development. Strong clonal differences in the amount and timing of conelet drop have been observed (Sweet and Thulin 1969, Forbes 1971). Bramlett (1972) observed a 3 to 65 per cent survival rate in two consecutive years, and Brown (1971) recorded 20 to 52 per cent survival. Conelet drop appeared to be more common in lower than upper portions of the crown (Sweet and Thulin 1969) and on colder aspects of the tree (Brown 1970). Abscission of unpollinated seed conelets has been studied anatomically and phenologically but not physiologically (Sweet 1973). Unpollinated Pinus conelets develop normally for several weeks, then 80 to 90 per cent abscise within a period of two weeks. This may be triggered by failure of ovules to develop.

There are some references to abscission of Pinus cones in the spring, one year after pollination at the time of rapid cone growth (McWilliam 1959a, Katsuta and Satoo 1964, Krugman 1970, Sweet and Bollmann 1971). Katsuta and Satoo (1964) concluded that cone drop at this time had no connection with the proportion of fertilized ovules or embryo development. Sweet and Bollmann (1970) demonstrated that competition for carbohydrates and mineral nutrients may result in premature cone abscission in P. radiata. Auxin diffusing from germinating pollen in Pinus might stimulate increased auxin production by the ovule. This would attract metabolites, reducing ovule abortion and cone abscission. Experiments applying auxin to cut pedicels of cones demonstrate that high auxin levels from cones prevent abscission (Sweet 1973).

There have been few studies of physiological changes associated with cone loss. White and Knopp (1978) showed that ATP levels dropped as conelets aborted. Competition for water may affect cone drop (Rehfeldt et al. 1971) but, in P. palustris, fertilizer application and irrigation did not reduce conelet abortion (Summerville et al. 1979). Low temperatures may also cause cone drop (Sweet and Thulin 1969). Katsuta (1975) studied cone development in P. thunbergii and P. densiflora in response to chilling and daylength but no data were given on cone loss. Cone position in the crown may have an effect (Brown 1973). Fungal and insect agents have also been suggested (Wright 1953).

Postfertilization cone loss has been reported (Brown 1970, Bramlett 1972) but is not common. Rehfeldt et al. (1971) described postfertilization cone drop in P. monticola and showed it to be influenced by early summer water deficit. Sweet (1973) suggested the low cone loss at this time is due to lessened competition for nutrients, because cones and seeds have approached full size by the time of fertilization. It has been observed that the seed coat is well developed at fertilization and seeds and seed wings have usually separated from the ovuliferous scale. No vascular connection exists between seed and ovuliferous scale in most conifers but, if present, it is rudimentary and not intact after fertilization (Owens et al. 1982, Singh and Owens 1981a, b, 1982). Consequently, seeds are usually autonomous during postfertilization development and no longer serve as strong metabolic sinks. Shoot elongation, also a strong metabolic sink, is generally complete by this time (Owens 1984b) and no longer competes with maturing cones for nutrients.

Seed maturation in conifers

Development of the conifer seed coat is described by Singh (1978). The seed coat (testa) usually begins to differentiate into a three-layered structure before fertilization. Differentiation of the thin outer sarcotesta, the thick, hard, middle sclerotesta and the thin, inner endotesta continues throughout embryo development (Singh and Johri 1972, Singh 1978). Only Cedrus and Cephalotaxus have a vascularized testa (Roy Chowdhury 1961, Singh 1961). Development of resin cavities in the seed coat has been described for several species of Abies (Owens and Molder 1977d, Singh and Owens 1981b, 1982). Resinous seeds pose particular problems in seed extraction and handling.

Changes occur within the female gametophyte after fertilization. A corrosion cavity forms by a breakdown of cells in the central portion of the female gametophyte. The young embryo is pushed into this cavity by elongating suspensor cells. In the few members of the Pinaceae in which this has been studied, a cavity forms even when fertilization does not occur (Singh and Owens 1981b, 1982). Therefore, cavity formation may be caused by the release of substances from the degenerating archegonia (J.N. Owens, pers. observation). The presence of a cavity may give the false impression that an embryo has formed but aborted.

Changes within the female gametophyte occur during embryo development. It undergoes some cell division, which may be diffuse or concentrated in certain areas, but the most conspicuous change is in the deposition of reserve food as fat, starch, and protein. One of the most complete studies of starch, lipid, and lipoprotein changes has been in the gametophyte of the primitive gymnosperm *Ginkgo* (Favre-Duchartre 1956, 1958a). Similar changes appear to occur in the gametophytes of the Pinaceae (Hakansson 1956, Takao 1960, Owens et al. 1982, Singh and Owens 1981a, b, 1982), but detailed histochemical and ultrastructural studies have not been made during conifer seed development. Hakansson (1956) followed embryo development and the state of food materials in the female gametophytes of *Pinus* and *Picea*. Some differences occur between the two genera but more complete studies are needed. A detailed ultrastructural study has been made of *P. sylvestris* embryos and female gametophytes in dry and germinating seeds (Simola 1974).

Seed wings develop in most conifers either from the ovuliferous scale (e.g. Pinaceae) or from the ovules (e.g. Cupressaceae). Details of seed wing development and the separation layer which forms between the ovuliferous scale and seed wing have been described for *Pseudotsuga* (Owens and Smith 1965). Factors affecting seed and wing abscission have not been studied, but complete separation does not always occur (J.N. Owens, pers. observation). The origin of seed wings from ovules has been described for *Thuja plicata* and *Chamaecyparis nootkatensis* (Owens and Molder 1980b, 1984a).

Embryogeny of hardwoods

Early embryogeny of angiosperms has been variously separated into three (Crété 1963), six (Johansen 1950), or more types. The systematic embryology of Davis (1966) uses Johansen's classification and most of the north temperate hardwoods fall into three major types. Most members of a family have the same type of development. Therefore, genera which have not yet been studied (e.g. *Fagus*, *Betula*) likely show embryogeny similar to other members of the same family. In all cases the first division of the zygote is by a transverse wall forming a terminal and a basal cell. Following this, considerable variation occurs. The Onagrad type occurs in *Acer*, *Quercus*, some *Populus* species, and some members of the Ulmaceae. In this type the terminal cell divides longitudinally and the basal cell has only a minor role in embryo development. *Alnus*, *Carya*, *Juglans*, most members of the Salicaceae, and all members of the Rosaceae have the Asterad type. This differs from the Onagrad type in that both the basal and terminal cells contribute to the embryo. *Ulmus*, and probably *Fraxinus*, have the Solanad type in which the terminal cell divides transversely and the basal cell divides to form a suspensor of two or more cells. There are no descriptions of the Platanaceae (Davis 1966).

Subsequent cell divisions are generally well coordinated but variations occur in different plants (Foster and Gifford 1974). A suspensor, consisting of a filament of variable numbers of cells, remains attached to the endosperm and elongates. Distal cells which have been forced into the endosperm divide in various directions to form a globular or heart shaped group of cells from which the embryo develops. Details of subsequent embryogeny of *Quercus* has been carefully studied (Stairs 1964). *Salix* (Maheshwari and Roy 1951) and *Populus* (Nagaraj 1952) have also been studied, but other genera have not. Therefore, it is not known if problems occur in embryogeny which might reduce seed production. It has been generalized that barriers to successful reproduction in angiosperms are primarily prezygotic, but some postzygotic selection does occur (Willson and Burley 1983). Double fertilization may increase relatedness of the endosperm to the zygote, thereby decreasing embryo abortion and postzygotic selection (Charnov 1979).

Polyembryony occurs in angiosperms but it has been rarely reported in hardwood forest trees. There are different types of polyembryony. True polyembryony occurs when more than one embryo arises within an embryo sac. This can occur by budding or cleavage of the proembryo, from synergids or antipodal cells, or from the nucellus or integument. In Ulmus, antipodal cells may give rise to additional embryos but these do not survive (Guignard and Mestre 1966). Suspensor budding results in polyembryony in Acer (Davis 1966). Polyembryony of unspecified types has been reported for Alnus, Juglans, Fraxinus, and Populus (Davis 1966); however, the frequency is low 0.71 to 7 per cent in Fraxinus) (Davis 1966) and is of less significance than in conifers. False polyembryony, embryos arising from different embryo sacs in the same ovule or fusion of two or more nucelli, occurs in some angiosperms but has not been reported in hardwoods. Polyembryony has also been used to describe the development of two ovules per ovary in Betula pubescens where, typically, only one ovule and embryo mature per fruit (Sulkinoja and Valanne 1980).

Agamospermy, the development of viable seeds in the absence of pollen, can occur in some hardwoods, e.g. Acer saccharum (Gabriel 1967). However, in most species, unpollinated ovules abort during development. In Alnus, Betula, Quercus, and Corylus (Hagman 1970), ovule abortion may be caused by inhibition of selfed pollen tube growth due to incompatability mechanisms in the style. Unpollinated ovules in Alnus and Betula abort, but leave full-sized empty seeds (Hagman 1970). However, unlike some conifers, abortion of many ovules in these species does not cause developing fruit to abscise (Sweet 1973).

Agamocarpy, the development of fruits in the absence of pollination, occurs in several hardwoods including Alnus, Betula (Hagman 1970), Fagus sylvatica (Nielsen and Schaffalitzky de Muckadell 1954), and Acer saccharum (Gabriel 1967). In many fruit trees fertilization occurs early in the reproductive cycle and provides the stimulus necessary for both seed and fruit development (Luckwill 1959). It is uncertain how common this is in north temperate hardwoods.

Ovule, flower, and fruit loss

Hardwood flowers have varying numbers of ovules depending upon the species. In some species all ovules can develop, whereas in others all but one abort. Six ovules typically develop in the ovary of Quercus, but only one normally matures into a seed (Mogensen 1975). In Betula only one of four ovules develops into seed (Clausen 1973a). In Populus trichocarpa, ovaries contain 50 to 70 ovules but only 25 to 40 are potentially functional. The others stop development early and abort (Stettler and Bawa 1971). Although ovule abortion decreases potential seed production, it may be an important method of selection or of insuring the survival of the remaining embryo(s) by decreasing competition for nutrients (Stephenson 1980).

Premature abscission of flowers is common in hardwood forest trees but the causes are uncertain (Sweet 1973). Williamson (1966) reported that 90 per cent of flowers initiated in Quercus alba abscised prematurely during the 4 months after anthesis. Sixty-five per cent of the abscission occurred between anthesis and fertilization and the remainder occurred during fertilization, embryo development, and acorn maturation. Comparable abscission was reported in a European Quercus and Tectona grandis (Bryndum and Hedegart 1969). Premature abscission has been related to damage from frost, drought, high temperatures, insects, fungi, birds, and mammals. A complete recent review of flower and fruit abortion, their causes, and ultimate functions is given by Stephenson (1981).

Some species have been studied more extensively than others. Eucalyptus pilularis shows considerable preanthesis flower abortion and abscission. This was correlated with major increases in rate of bud growth which implies a competition for metabolites (Florence 1964). Fruit abortion in response to limited resources was described for Catalpa speciosa as a trade-off between seed number and seed quality that permitted the parent plant to match fruit production with available resources (Stephenson 1980). Considerable research has been done on orchard species where immature flower and fruit drop are very noticeable and important. The work of Luckwill (1959, 1970) indicates that the developing ovule after pollination is a rich source of auxin and other plant hormones and, that if sufficient ovules develop, hormone levels are high enough in the fruit to prevent fruit abscission. The concepts developed by Luckwill (1970) concerning nutrients, hormones, and transport of nutrients in apple fruit abscission may also apply to hardwood forest trees but, as yet, there appears to be no comparable detailed information for the latter.

Seed maturation in hardwoods

Development of angiosperm seeds is described by Bhatnagar and Johri (1972) and briefly by Krugman et al. (1974). The seed coat is more variable and complex than in conifers, partially because it develops from a double integument in most angiosperms. Seed coat development has been described for several families but not for hardwood forest trees. Generally, the seed coat consists of more than three distinct layers which vary in structure and thickness. Unlike conifers the developing seed remains attached to the ovary wall by the funiculus, which leaves a scar, the hilum, on the seed coat when the seed abscises. Seed coats may be very thin (*Populus*, *Salix*), thick (*Crataegus*, *Eucalyptus*), membranous (*Ulmus*), or fleshy (*Magnolia*). Sometimes the seed coat bears hairs (*Populus*, *Salix*). In many, the wall of the fruit and the seed coat fuse (*Quercus*, *Platanus*, *Juglans*); in some, the pericarp forms a single wing (*Acer*, *Fraxinus*), two wings (*Alnus*, *Betula*), or a large membranous wing around the seed (*Ulmus*). For general descriptions of seed structure a manual such as Schopmeyer (1974) should be consulted.

In most hardwood forest trees, the endosperm is nearly or entirely consumed during embryo development and the seed contains a large embryo with large cotyledons in which food is stored (e.g. *Quercus*). In a few species the endosperm constitutes a large portion of the seed and the cotyledons are small (e.g. *Diospyros*) (Schopmeyer 1974). In most species embryos attain their full size by the time the seed or fruit ripens and is shed. In only a few (e.g. *F. nigra*) are embryos rudimentary or immature when seeds are shed.

Fruit development

The fruit is the seed containing structure that originates from the enlargement and modified development of the gynecium, the gynecium being one or more fused carpels (Foster and Gifford 1974). Other parts of the flower may also form part of the mature fruit but generally not in hardwood forest trees. The fruits of most hardwoods are simple, developing from a single ovary. They are dry dehiscent (e.g. *Populus*, *Salix*), dry indehiscent achenes (e.g. *Platanus*), samaras (e.g. *Acer*, *Fraxinus*, *Ulmus*), or nuts (e.g. *Quercus*, *Juglans*, *Fagus*).

Growth of the fruit is usually dependent on stimulation provided by pollination and subsequent fertilization. However, some plants may develop seedless fruits without pollination, or after embryo abortion. Fruits of this kind are termed parthenocarpic. Parthenocarpy has been divided into three types: (1) fruits which develop without pollination; (2) fruits in which pollination stimulates development, but fertilization of ovules does not occur; and, (3) fruits in which fertilization occurs but abortion of embryos takes place before fruit maturation (Leopold 1964). Parthenocarpy in hardwood forest trees is poorly understood.

Summary, and recommendations for future research

The embryogeny of conifers is well described, whereas there have been few studies of embryogeny of hardwood forest trees. More important, however, are studies of embryogeny designed to determine possible causes of ovule and embryo abortion. It is generally thought that incompatability-barriers to reproduction in angiosperms occur during the prezygotic stages and those of gymnosperms occur postzygotically. However, the literature to support this hypothesis is limited in forest trees. In hardwoods there are many cases of ovule abortion after the time of fertilization, but studies must be made to determine if fertilization actually has occurred. Similarly, in conifers ovule abortion can be very high before fertilization as well as after. The causes of abortion at these times must be determined. The ability to induce flowering on small potted physiological clones (rooting cuttings) provides a valuable tool in these studies since reproductive plants can be grown in controlled environments where endogenous and exogenous factors can be tested.

The causes of flower and conelet abortion should be determined in a similar manner for most species. One exception which has been extensively studied is cone drop in Pinus due to inadequate pollination. Factors such as low temperature, moisture stress, nutrition, and competition for nutrients between vegetative and reproductive structures should be examined under controlled conditions. Physiological changes associated with ovule, flower, and cone abortion should also be studied. Species for which the normal development is known should be studied first and the normal development should be an integral part of any ecological or physiological study.

The relationship between embryo and endosperm or female gametophyte during seed development is poorly understood for both hardwoods and conifers. Studies of these physiological changes may be important in understanding normal seed development and abortion.

Growth of trees in seed orchards allows for some control of environmental and cultural conditions which adversely affect seed production in nature. An understanding of embryo, seed, cone, and flower development is a first essential step in determining causes of seed, cone, and flower losses. Such studies could result in significant increases in seed quantity and quality.

SYNOPSIS

Seed production of north temperate forest trees is a broad subject because of the diversity of conifer and hardwood species, their long and often complex life cycles, and the many approaches needed to fully understand causes of variable seed production. Ideally, factors affecting seed production of all species should be investigated in a comprehensive fashion but this is not usually economically feasible. The task is made easier because: (1) particular aspects of development are especially important in influencing seed production; and, (2) many species are reproductively similar, so cautious generalizations may be made from intensive studies of species having representative reproductive development.

Included in the "Summary and Recommendations for Future Research" sections at the end of each chapter are many major and minor problems which should be addressed. From these, three major areas are recommended most highly for research because: (1) their solutions could potentially lead to significant gains in seed production; (2) the technology is available to solve these problems; and, (3) there is adequate information on commercially important species. These areas are floral induction, pollination, and seed losses due to cone, ovule, and embryo abortion.

Floral induction

Poor floral initiation is often the primary cause for poor seed production. Floral induction has been achieved in many conifers but few hardwoods. Further research is required to develop more consistent results with shorter treatments. The major shortcomings of much of the early research were the lack of carefully controlled experimental conditions, the use of nonuniform plant material, and the inclusion of too many variables in experiments. Future research should utilize smaller potted trees grown in controlled environments; rooted cuttings would provide genetically uniform material. In all experiments treatments should be correlated with easily recognizable developmental stages (e.g. per cent shoot elongation).

Promising cone induction techniques include $GA_{4/7}$ with high moisture stress and high temperatures in the Pinaceae, and GA_3 alone in the Cupressaceae and Taxodiaceae However, more research is needed to refine these techniques, reduce treatment times, and regulate the number and sex of induced cones. The use of small clonal material grown in controlled environments will be essential for consistent results. If field grown trees are used, careful monitoring of moisture stress and correlation of treatments with shoot development will be necessary. There is now adequate literature to show that the most consistent results are obtained when treatment times, methods, and growing conditions are carefully controlled. Basic research is needed to determine how induction treatments affect the biochemistry, growth, and development of trees. The role of growth regulators in cone induction should be determined.

Comparable studies using hardwoods should begin using controlled conditions and genetically uniform material rather than field grown trees. In general, the approaches to be used for hardwoods may parallel those found to be successful in conifers, but care should be taken to avoid errors made in many early conifer cone induction studies.

Successful floral induction should be followed by studies of subsequent cone or fruit development in order to determine optimal numbers of cones or fruits for maximum seed production and highest quality of seeds and seedlings. This is essential in order to demonstrate that floral induction is a sound practice, worthy of the investment.

Floral induction research, so far, indicates that the use of small potted trees in containerized seed orchards, utilizing small areas and controlled conditions, may be a viable alternative to conventional seed orchards for seed production of some species. However, the cost effectiveness of this practice should be determined using one of the more extensively studied species (e.g. *Picea glauca*).

Pollination

A second major area of research is pollination, since poor pollination is a common cause of poor seed production. Pollination at the right time with high quality pollen can significantly increase seed production.

Supplemental mass pollinations will likely be necessary in most seed orchards. Therefore, research must continue on pollen forcing, collection, storage, and viability testing. Pollen viabilty must be related to pollen fertility through studies of seedset. Basic studies of pollen physiology are essential background for all pollen studies but are lacking of most forest trees. Pollination techniques and optimal times of pollination for maximum seed production must be determined. In many genera, species are very similar so detailed studies may not be required for all species. Many aspects of pollination will be most effectively studied using small florally induced trees grown in controlled environments.

Seed losses due to cone, ovule, and embryo abortion

The loss of cones, fruits, ovules, embryos, or seeds can be significant in most forest trees but the causes are often unknown and have been studied very little. Possible causes include incompatabilities at the pre- and post-fertilization stages; the inherent ability of the parent tree to carry cones, fruits, or seeds to maturity; competition between vegetative and reproductive development; and, environmental effects on cone, flower, fruit, ovule, embryo, and seed development. Studies determining the causes of these losses are needed and would be most effectively carried out using small florally induced trees grown in controlled environments.

Concluding remarks

Much of the technology is now available to significantly increase seed production in many forest trees. However, further research is required to refine the technology and provide basic information about tree reproductive physiology and development.

The breadth of any problem is often more than can be handled by one individual or laboratory. However, developmental, physiological, biochemical, and ecological aspects should be investigated using current techniques. This often involves specialized and complex equipment and techniques. Therefore, cooperative efforts between research groups should be encouraged. The cooperative efforts of Drs. Pharis, Ross, Webber, and Owens on cone induction in western Canada is an example of the benefits of collaboration on biochemical, physiological, and developmental aspects of conifer reproduction. Other regional research groups should be encouraged.

Supporting agencies must recognize that seed production research is long term, requiring a continuing commitment on the part of supporting agencies and the researcher. If research is intermittent, the investments will be, for the most part, lost. Upon completion of projects, the results should be published in accessible journals in order to keep useful information from becoming lost in internal or progress reports. There should also be more emphasis on technology transfer from researchers to those working in seed production.

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LITERATURE CITED

Adams, W.T. 1982. Clonal variation in pollen-related characteristics of Douglas-fir. Can. J. Forest Res. 12: 403-408.

Adams, W.T.; Joly, R.J. 1980. Allozyme studies in loblolly pine seed orchards: clonal variation and frequency of progeny due to self-fertilization. Silvae Genet. 29: 1-4.

Ager, A.A.; Guries, R.P. 1982. Barriers to interspecific hybridization in *Ulmus americana*. Euphytica 31: 909-920.

Ahlgren, C.E. 1957. Phenological observations of nineteen native tree species in Northern Minnesota. Ecology 38: 622-628.

Ahlgren, C.E. 1972. Some effects of inter- and intraspecific grafting on growth and flowering of five-needle pines. Silvae Genet. 21: 122-126.

Alam, M.T.; Grant, W.F. 1971. Pollen longevity in birch (*Betula*). Can. J. Bot. 49: 497-498.

Allen, G.S. 1946. Embryogeny and development of the apical meristems of *Pseudotsuga*. I. Fertilization and early embryogeny. Am. J. Bot. 33: 666-676.

Allen, G.S. 1963. Origin and development of the ovule in Douglas-fir. Forest Sci. 9: 386-393.

Allen, G.S.; Owens. J.N. 1972. The life history of Douglas-fir. Dep. Environ. Can., Can. Forest Serv. Ottawa. 139 p.

Allen, P.H.; Trousdell, K.B. 1961. Loblolly pine seed production in the Virginia - North Carolina coastal plain. J. For. 59: 187-190.

Allen, R.M. 1953. Release and fertilization stimulate longleaf pine cone crop. J. For. 51: 827.

Anderson, N.F.; Guard, A.T. 1964. A comparative study of the vegetative transitional and floral apex of *Acer pseudoplatanus* L. Phytomorphology 14: 500-508.

Andersson, E. 1965. Cone and seed studies in Norway spruce (*Picea abies* (L.) Karst.). Stud. For. Suec. 23: 1-214.

Andersson, E. 1980. Temperature-conditioned irregularities in pollen mother cells of *Picea abies* (L.) Karst. Hereditas 92: 27-35.

Anon. 1957a. Induction of flowering in *Pinus radiata*. Rep. For. Timb. Bur. Aust. 1956. 25 p.

Anon. 1957b. Douglas-fir cones increased in United States experiments. B.C. Lumberman 41: 16.

Argus, G.W. 1974. An experimental study of hybridization and pollination in *Salix* (willow). Can. J. Bot. 52: 1613-1619.

Asakawa, S.; Fujita, K. 1966. Studies on the management of seed stands. (I) The establishment of experimental seed stands of *Pinus densiflora* and *Larix leptolepis* and the results obtained for three years (1962 to 1964). Forest Exper. Stn., Meguro, Tokyo Bull. No. 184: 81-134.

Azniev, Ju. N. 1970. [Acceleration of the onset of sexual maturity of *Pinus sylvestris* under the influence of lupin]. Lesn. Z. 13: 165-167.

Bagnell, C.R., Jr. 1975. Species distinction among pollen grains of *Abies*, *Picea*, and *Pinus* in the rocky mountain area (A scanning electron microscope study). Rev. Paleobot. Paleon. 19: 203-220.

Baird, A.M. 1953. The life history of *Callistris*. Phytomorphology 3: 258-284.

Baradat, Ph. 1967. Etudes preliminaires sur la fructification du Douglas. Rev. For. Française 11: 698-713.

Barber, J.C.; Stewart, D.M. 1957. Vacuum storage of pollen proves feasible. Minn. Forestry Note No. 62. 2 p.

Barendse, G.W.M.; Rodriques Pereira, A.S.; Berkers, P.A.; Driessen, F.M.; Van Eyden-Emons, A.; Linskens, H.F. 1970. Growth hormones in pollen, styles and ovaries of *Petunia hybrida* and of *Lilium* species. Acta Bot. Néerl. 19: 175-186.

Barker, P.A.; Freeman, D.C.; Harper, K.T. 1982. Variation in the breeding system of *Acer grandidentatum*. Forest Sci. 28: 563-572.

Barner, H.; Christiansen, H. 1960. The formation of pollen, the pollination mechanism, and the determination of the most favorable time for controlled pollination in *Larix*. Silvae Genet. 9: 1-11.

Barnes, B.V. 1969. Effects of thinning and fertilizing on production of western white pine seed. USDA Forest Serv. Res. Paper No. INT-58. 14 p.

Barnes, R.L.; Bengston, G.W. 1968. Effects of fertilization, irrigation, and cover cropping on flowering and on nitrogen and soluble sugar composition of slash pine. Forest Sci. 14: 172-180.

Barnes, B.V.; Bingham, R.T. 1963. Flower induction and stimulation in western white pine. USDA Forest Serv. Res. Paper No. INT-2. 10 p.

Barnes, R.D.; Mullin, L.J. 1974. Flower phenology and productivity in clonal seed of *Pinus patula*, *P. elliotti*, *P. taeda*, and *P. kesiya* in Rhodesia. Rhodesia Forest Comm. Res. Div., Res. Pap. No. 3. 81 p.

Bassett, I.J.; Crompton, C.W.; Parmalee, J.A. 1978. An atlas of airborne pollen grains and common fungus spores of Canada. Can. Dep. Agric. Res. Branch, Monograph No. 18. 321 p.

Beers, W. 1974. Industry's analysis of operational problems and research in increasing cone and seed yields. Pages 86-96 *in* J. Kraus, ed. Seed yield from southern pine seed orchards. Ga. Forest Res. Council, Macon, Ga.

Beers, W.L., Jr.; Bivens, J.; Micha, J.E. 1981. Pollen collection. Pages 30-32 *in* Franklin, E.C. ed. Pollen management handbook. USDA Forest Serv. Agric. Handbook No. 587.

Beetle, A.A. 1974. Range survey in Teton County, Wyoming. Part 4: Quaking aspen. Wyoming Agr. Exper. Stn. Sci. Mono. 27: 2-28.

Beineke, W.F.; Masters. C.J. 1976. Controlled pollination in black walnut. Pages 66-72 *in* Proc. Tenth Central States Forest Tree Improv. Conf.

Bell, H.K.; Childers, N.F. 1954. Peach nutrition. *In* Childers, N.F., ed. Mineral nutrition of fruit crops. Hort. Publ., Rutgers Univ., New Brunswick, N.J.

Bengston, G.W. 1969. Growth and flowering of clones of slash pine under intensive culture: early results. USDA Forest Serv. Res. Paper No. SE-46, 9 p.

Benoit, L.F.; Skelly, J.M.; Moore, L.D. 1983. The influence of ozone on *Pinus strobus* L. pollen germination. Can. J. Forest Res. 13: 184-187.

Bergman, F.; Kardell, L. 1975. [An experiment in flower induction in *Pinus sylvestris*.] Foreningen skogstradsforadling Institutet for skogsforbattring arsbok. p. 79-94.

Berlyn, G.P. 1962. Developmental patterns in pine polyembryony. Am. J. Bot. 49: 327-333.

Bernier, G. 1971. Structural and metabolic changes in the shoot apex in transition to flowering. Can. J. Bot. 49: 803-819.

Bhatnagar, S.P.; Johri, B.M. 1972. Development of angiosperm seeds. Pages 77-149 *in* Kozlowski, T.T., ed. Seed biology. Vol. I. Academic Press, New York.

Bilan, M.V. 1960. Stimulation of cone and seed production in pole-size loblolly pine. Forest Sci. 6: 207-220.

Binder, W.D.; Ballantyne, D.J. 1975. The respiration and fertility of *Pseudotsuga menziesii* (Douglas-fir) pollen. Can. J. Bot. 53: 819-823.

Binder, W.D.; Mitchell, G.M.; Ballantyne, D.J. 1974. Pollen viability, testing, storage, and related physiology. Review of the literature with emphasis on gymnosperm pollen. Dep. Environ., Can.

Forest Serv., Pacific Forest Res. Centre Rep. BC-X-105. 37 p.

Bingham, R.T.; Wise, K.C. 1968. Western white pine cones pollinated with 1- to 3-year-old pollen gives good seed yields. USDA Forest Serv. Res. Note No. INT-81. 3 p.

Bingham, R.T.; Hoff, R.J.; Steinhoff, R.J. 1971. Genetics of western white pine. USDA Forest Serv. Res. Paper WO-12.

Bingham, W.E.; Krugman, S.L.; Estermann, E.F. 1964. Acrylamide electrophoresis of pine pollen proteins. Nature 202: 923-924.

Bleymuller, H. 1976. Investigations on the dependence of flowering in Norway spruce (Picea abies (L.) Karst) upon age. Acta Hort. 56: 169-172.

Bleymuller, H. 1978. Versuche mit Fichtenpfropflingen zur Blühinduktion durch Wirkstoffbehandlung. Silvae Genet. 27: 117-122.

Bonnet-Masimbert, M. 1971. Induction florale précoce chez Cupressus arizonica et Chamaecyparis lawsoniana. Silvae Genet. 20: 53-100.

Bonnet-Masimbert, M. 1982. Effect of growth regulators, girdling and mulching on flowering of young European and Japanese larches under field conditions. Can. J. Forest Res. 12: 270-279.

Bonnet-Masimbert, M.; Delanzy, P.; Chanteloup, G.; Coupaye, J. 1982. Influence de l'état d'activité des racines sur la floraison induite par des gibbérellines 4 et 7 chez Pseudotsuga menziesii (Mirb.) Franco. Silvae Genet. 31: 178-183.

Bower, D.R.; Smith, J.L. 1961. Partial girdling multiplies shortleaf cones. USDA Forest Service, Southern Forest Exper. Stn. Forestry Note No. 132.

Boyer, W.D. 1973. Air temperature, heat sums, and pollen shedding phenology of longleaf pine. Ecology 54: 420-426.

Boyer, W.D. 1978. Heat accumulation: an easy way to anticipate the flowering of southern pines. J. For. 76: 20-23.

Boyer, W.D. 1981. Pollen production and dispersal as affected by seasonal temperature and rainfall patterns. Pages 2-9 in Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv. Agric. Handbook 587.

Boyer, W.D.; Woods, F.W. 1973. Date of pollen shedding by longleaf-pine advanced by increased temperatures at strobili. Forest Sci. 19: 315-318.

Bramlett, D.L. 1972. Cone crop development records for six years in shortleaf pine. Forest Sci. 18: 31-33.

Bramlett, D.L. 1973. Pollen phenology and dispersal pattern for shortleaf pine in the Virginia Piedmont. USDA Forest Serv. Res. Paper No. SE-104. 7 p.

Bramlett, D.L. 1981. Effectiveness of wind pollination in seed orchards. Pages 10-14 in Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv. Agric. Handbook No. 587.

Bramlett, D.L.; Belanger, R.P. 1976. Fertilizer and phenotypic selection increase growth and flowering of young Virginia pine. Forest Sci. 22: 461-467.

Bramlett, D.L.; O'Gwynn, C.H. 1981. Controlled pollination. Pages 44-51 in Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv. Agric. Handbook No. 587.

Bramlett, D.L.; Belcher, E.W.; DeBarr, G.L.; Hartel, G.D.; Karrfalt, R.P.; Lantz, C.W.; Miller, T.; Ware, K.D.; Yates, H.O. 1977. Cone analysis of southern pines. A guidebook. USDA Forest Serv. Gen. Tech. Rep. No. SE-13. 28 p.

Brazeau, M.; Veilleux, J.M. 1976. Bibliographie annotée sur les effets de la fertilization sur la production de cones et de semences. Québec, Ministere des Terres et Forêts, Service de la Recherche, Mémoire 25. 26 p.

Bridgwater, F.E.; Trew, I.F. 1981. Supplemental mass pollination. Pages 52-57 in Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv. Agric. Handbook No. 587.

Brinkman, K.A. 1962. Fertilizers increase seed production of shortleaf pine in Missouri. Tree Planter's Notes 53: 18-19.

Brix, H.; Portlock, F.T. 1982. Flowering response of western hemlock seedlings to gibberellin and water stress treatments. Can. J. Forest Res. 12: 76-82.

Bróndo, P. 1970. Flowering of forest trees. Transl. For. Comm., Lond. No. 434, 1-11. Transl. by Gombrich, L. from Tidsskr. Skogbr. 1968. 76: 179-190.

Brown, I.R. 1970. Premature cone loss in grafted clones of Scots pine. *In* Sexual reproduction of forest trees. Vol. I. 15 p. Finnish Forest Res. Inst., Helsinki.

Brown, I.R. 1971. Flowering and seed production in grafted clones of Scots pine. Silvae Genet. 20: 121-132.

Brown, I.R. 1973. Factors affecting the yield from Scots pine seed orchards. Scot. For. 27: 296-307.

Brune, A. 1973. Physiology of flowering in *Cupressus arizonica* Greene seedlings. Ph.D. Dissertation, Univ. of Florida, Gainesville, Fla. 64 p.

Bryndum, K.; Hedegart, T. 1969. Pollination of teak. Silvae Genet. 18: 77-80.

Buchholz, J.T. 1926. Origins of cleavage polyembryony in conifers. Bot. Gaz. 81: 55-71.

Buchholz, J.T. 1929. The embryogeny of the conifers. Pages 359-392 *in* Proc. Fourth Internat. Bot. Congr.

Buchholz, J.T. 1931. The pine embryo and the embryos of related genera. Trans. Ill. Acad. Sci. 23: 117-125.

Buchholz, J.T. 1939a. The morphology and embryogeny of *Sequoia gigantea*. Am. J. Bot. 26: 93-101.

Buchholz, J.T. 1939b. The embryogeny of *Sequoia sempirvirens* with a comparison of the *Sequoias*. Am. J. Bot. 26: 248-257.

Buchholz, J.T. 1941. Embryogeny of the Podocarpaceae. Bot. Gaz. 103: 1-37.

Buchholz, J.T. 1944. The cause of sterility in cross-pollinations between certain species of pines. Am. J. Bot. 31: Suppl. 2S.

Buchholz, J.T. 1946. Volumetric studies of seeds, endosperms, and embryos in *Pinus ponderosa* during embryonic differentiation. Bot. Gaz. 108: 232-244.

Buchholz, J.T. 1950. Embryology of gymnosperms. Pages 375-376 *in* Proc. Seventh Internat. Bot. Congr., Stockholm.

Buell, M.F. 1947. Mass dissemination of pine pollen. J. Elisha Mitchell Sci. Soc. 63: 163-167.

Burdon, R.D.; Low, C.B. 1973. Seed production of *Pinus radiata* clones on four different sites. N.Z.J. For. Sci. 3: 211-219.

Cade, S.C.; Hsin, L.Y. 1977. Girdling: its effect on seed-cone bud production, seed yield, and seed quality in Douglas-fir. For. Res. Tech. Rep. Seed Orchard Res. W.042-3303/77/23., Weyerhauser Corp. 9 p.

Cade, S.C.; Jackson, K. 1976. Cone bud production of potted Douglas-fir grafts as influenced by cultural practices. For. Res. Tech. Rep., Weyerhauser Corp. 27 p.

Caesar, J.C.; MacDonald, A.D. 1983. Shoot development in *Betula papyrifera*. II. Comparison of vegetative and reproductive short-shoot growth. Can. J. Bot. 61: 3066-3071.

Callaham, R.Z.; Steinhoff, R.J. 1966. Pine pollens frozen five years produce seed. USDA Forest Serv. Res. Paper No. NC-6: 94-101.

Camefort, H. 1968. Sur l'organisation du néocytoplasme dans les proembryons tetranclées du *Larix decidua* Mill. (*Larix europea* D.C.) et l'origine des mitochondries et des plastes de l'embryon chez cette espèce. C.R. Acad. Sc. Paris Série D. 266: 88-91.

Camefort, H. 1969. Fecondation et proembryogénèse chez les Abiétacées (notion de neocytoplasme). Rev. Cytol. Biol. Végét. 32: 253-271.

Camefort, H. 1978. Evolution des structures cytoplasmiques au cours de la gamétogénèse male chez le Picea asperata. Soc. Bot. Fr. Act. Bot. No. 1-2: 109-113.

Carlson, M.; Hsin, L. 1976. Comparative effectiveness of two controlled pollination techniques in Douglas-fir. For. Res. Tech. Rep. 50-3201/76/19. Weyerhauser Corp.

Carson, M.J.; Sweet, G.B.; Hong, S.O. 1977. Gibberellin-induced effects on initiation and development of long shoot primordia on some seed orchard grafts. N.Z. For. Serv., Tree Imp. Internal. Rep. No. 133. 12 p.

Cayford, J.H.; Jarvis, J.M. 1967. Fertilizing with ammonium nitrate improves red pine seed production. J. For. 65: 402-403.

Cecich, R.A. 1981. Applied gibberellin $A_{4/7}$ increases ovulate strobili production in accelerated growth jack pine seedlings. Can. J. Forest Res. 11: 580-585.

Cecich, R.A. 1983. Flowering in a jack pine seedling seed orchard increased by spraying with gibberellin $A_{4/7}$. Can. J. Forest Res. 13: 1056-1062.

Cecich, R.A. 1984. The histochemistry and ultrastructure of jack pine microsporangia during the winter. Am. J. Bot. 71: 851-864.

Chailakhyan, M.H. 1936a. [On the mechanism of plant development.] C.R. (Doklady) Acad. Sci. U.R.S.S. 10: 89-93.

Chailakhyan, M.H. 1936b. [On the hormonal theory of plant development.] C.R. (Doklady) Acad. Sci. U.R.S.S. 12: 443-447.

Chailakhyan, M.H. 1936c. [New facts in support of the hormonal theory of plant development.] C.R. (Doklady) Acad. Sci. U.R.S.S. 13: 79-83.

Chailakhyan, M.H. 1937. [Concerning the hormonal nature of plant development processes.] C.R. (Doklady) Acad. Sci. U.R.S.S. 16: 227-230.

Chailakhyan, M.Kh. 1961. Effect of gibberellins and derivatives of nucleic acid metabolism on plant growth and flowering. Pages 531-542 in Proc. Fourth Internat. Conf. Plant Growth Regulators. Iowa State Univ. Press., Ames, Iowa.

Chailakhyan, M.Kh. 1968. Flowering hormones of plants. Pages 1317-1340 in Wightman, E.F.; Setterfield, G.S., eds. Biochemistry and physiology of plant growth substances. Runge Press, Ottawa.

Chalupka, W. 1978. Effect of growth regulators on the flowering of Scots pine (Pinus sylvestris L.) grafts. Silvae Genet. 27: 62-65.

Chalupka, W. 1979. Effect of growth regulators on flowering of Norway spruce (Picea abies (L.) Karst.) grafts. Silvae Genet. 28: 125-127.

Chalupka, W. 1980. Regulation of flowering in Scots pine (Pinus sylvestris L.) grafts by gibberellins. Silvae Genet. 29: 118-121.

Chalupka, W. 1981. Influence of growth regulators and polythene covers on flowering of Scots pine and Norway spruce grafts. Silvae Genet. 30: 142-146.

Chalupka, W.; Giertych, M. 1977. The effect of polyethylene covers on the flowering of Norway spruce (Picea abies (L.) Karst) grafts. Arboretum Kornickie 22: 185-192.

Chamberlain, C.J. 1935. Gymnosperms: structure and evolution. University of Chicago Press, Chicago. (Reprint, 1966. Dover Pub. Inc. N.J.). 484 p.

Chandler, C.; Mavrodineanu, S. 1965. Meiosis in Larix laricina Koch. Contr. Boyce Thompson Inst. 23: 67-75.

Chandler, R.F., Jr. 1938. The influence of nitrogenous fertilizer applications upon seed production of certain deciduous forest trees. J. For. 36: 761-766.

Charnov, E.L. 1979. Simultaneous hermaphroditism and sexual selection. Proc. Nat. Acad. Sci. (USA) 76: 2480-2484.

Chen, T.H. 1981. [Testing of germinating capacity of tree pollens with TTC]. J. Nanjing Tech. Coll. For. Prod. No. 4: 116-119.

Chen, T.K.; Wang, F.H. 1978. Embryogeny of *Pseudotaxus chienii* in relation to its taxonomic position. Acta Bot. Sin. 16: 1-10.

Chesnoy, L. 1969. Sur la participation du gamète male à la constitution du cytoplasme de l'embryon chez le *Biota orientalis* Endl. Rev. Cytol. Biol. Végét. 32: 273-294.

Chesnoy L. 1973. Sur l'origine paternelle des organites du proembryon du *Chamaecyparis lawsoniana* A. Murr. (Cupressacées) Caryologia 25. Suppl.: 223-232.

Chesnoy, L. 1977. Etude cytologique des gamètes, de la fecondation et de la promebryogenèse chez le *Biota orientalis* Endl. III. Fecondation et proembryogenèse; transmission du cytoplasme du gamète male au proembryon. Rev. Cytol. Biol. Végét. 40: 293-396.

Chesnoy, L.; Thomas, M.J. 1971. Electron microscopy studies on gametogenesis and fertilization in gymnosperms. Phytomorphology 21: 50-63.

Ching, K.K. 1969. Pollen and seed: a good linkage for reforestation. Pages 19-21 *in* R.K. Herman, ed. Symp. Proc. Regeneration of ponderosa pine. Oregon State Univ., Sch. For., Corvallis, Ore.

Ching, T.M.; Ching, K.K. 1962. Physical and physiological changes in maturing Douglas-fir cones and seed. Forest Sci. 8: 21-31.

Ching, T.M.; Ching, K.K. 1964. Freeze-drying pine pollen. Plant Physiol. 39: 705-708.

Ching, T.M.; Ching, K.K. 1972. Content of adenosine phosphates and adenylate energy charge in germinating ponderosa pine seedlings. Plant Physiol. 50: 536-540.

Ching, T.M.; Ching, K.K. 1976. Rapid viability tests and aging study of some coniferous pollen. Can. J. Forest Res. 6: 516-522.

Ching, T.M.; Ranzoni, M.W.; Ching, K.K. 1975. ATP content and pollen germinability of some conifers. Plant Sci. Letters. 4: 331-333.

Chira, E. 1963. [A rapid method for determining the germinating qualities of pollen in some species of *Pinus*]. Biologie 18: 390-395.

Chira, E.; Berta, F. 1965. Eine der Ursachen der Nichtkreuzungsfahigkeit von Artun aus der Gattung *Pinus*. Biológia (Bratislava) 20: 600-609.

Chira, E.; Berta, F. 1978/79. [Sugars in the pollen of some coniferous species]. Acta Dendrobiologica No 1/2: 335, 337-359.

Christiansen, H. 1960. On the effect of low temperature on meiosis and pollen fertility in *Larix decidua* Mill. Silvae Genet. 9: 72-78.

Christiansen, H. 1969. On the pollen grain and fertilization mechanism of *Pseudotsuga menziesii* (Mirb. et Franco) var. *viridis* Schwer. Silvae Genet. 18: 97-107.

Chung, M.S. 1981. Flowering characteristics of *Pinus sylvestris* L. with special emphasis on the reproductive adaptation to local temperature factor. Acta Forest. Fenn. 169: 1-69.

Clausen, K.E. 1973a. The effect of pollen irradiation on reproductive capacity, seedling growth, and variation of *Betula nigra*. I. Seed yield, germination, and germinant abnormalities. Rad. Bot. 13: 47-54.

Clausen, K.E. 1973b. The effect of pollen irradiation on reproductive capacity, seedling growth, and variation of *Betula nigra*. II. Seedling growth and induced variation. Rad. Bot. 13: 259-268.

Coffen, D.O.; Bordelon, M.A. 1981. Stem breakage effect on cone and pollen production in *Pinus monticola* (Dougl.). USDA Forest Serv. Res. Note No. INT-312. 6 p.

Colangeli, A.; Owens, J.N. 1984. Pollination mechanism in western hemlock. Page 20 *in* Genetic Gain: Incorporation and Implications for Forestry. Proc. West. For. Gen. Ass. 1984 Annual Meeting.

Coleman, W.K.; Thorpe, T.A. 1978. In vitro culture of western red cedar (*Thuja plicata*). II. Induction of male strobili from vegetative shoot tips. Can. J. Bot. 56: 557-564.

Colwell, R.N. 1951. The use of radioactive isotopes in determining spore distribution patterns. Am. J. Bot. 38: 511-523.

Conrad, A.H. 1900. A contribution to the life history of *Quercus*. Bot. Gaz. 29: 408-418.

Cook, S.A.; Stanley, R.G. 1960. Tetrazolium chloride as an indicator of pine pollen germinability. Silvae Genet. 9: 134-136.

Cooley, J.H. 1970. Thinning and fertilizing red pine to increase growth and cone production. USDA Forest Serv. Res. Paper No. NC-42. 8 p.

Copes, D.L. 1973. Effect of annual leader pruning on cone production and crown development of grafted Douglas-fir. Silvae Genet. 22: 167-173.

Courtot, Y.; Baillaud, L. 1955. Sur la répartition des sexes chez un *Chamaecyparis*. Ann. Sci. Univ. Bescancon, 2°Ser. Bot. 6: 75-81.

Coutts, M.P.; Bowen, M.R. 1973. Flowering and seed production. British Forestry Comm. Ann. Rep. For. Res. p. 89-93.

Cram, W.H.; Lindquist, C.H. 1984. Pollen viability studies for *Picea pungens*. For. Chron. 60: 93-95.

Cresti, M.; Pacini, E.; Ciampolini, F.; Sarafatti, G. 1977. Germination and early tube development in vitro of *Lycopersicum peruvianum* pollen: ultrastructural features. Planta 136: 209-247.

Crété, P. 1963. Embryo. Pages 171-220 *in* Maheshwari, P., ed. Recent advances *in* the embryology of Angiosperms. Catholic Press, Ranchi, India.

Crocker, T.C., Jr. 1964. Fruitfulness of longleaf trees more important than culture in cone yield. J. For. 62: 822-823.

Crocker, T.C., Jr. 1973. Longleaf pine cone production in relation to site index, stand age and stand density. USDA Forest Serv. Res. Note No. SO-156.

Cumming, W.C.; Righter, R.I. 1948. Methods used to control pollination of pines in the Sierra Nevada of California. USDA, Forest Serv. Cir. No. 792. 18 p.

Curtis, J.D.; Popham, R.A. 1972. The developmental anatomy of long-branch terminal buds of *Pinus banksiana*. Am. J. Bot. 59: 194-202.

Dallimore, W.; Jackson, A.B. 1966. A handbook of Coniferae and Ginkgoaceae. Revised by Harrison, S.G. St. Martin's Press. N.Y. 729 p.

Daniels, J.D. 1978. Efficacy of supplemental mass-pollination in a Douglas-fir seed orchard. Silvae Genet. 27: 52-58.

Daubenmire, R. 1960. A seven-year study of cone production as related to xylem layers and temperature in *Pinus ponderosa*. Am. Mid. Nat. 64: 187-193.

Davis, G.L. 1966. Systematic Embryology of the Angiosperms. Wiley, New York. 528 p.

Debazac, E.F. 1965. Morphogenèse et sexualité chez les Pinacées. Acad. Soc. Lorraine Sci., Bull. 5: 212-228.

DeJong, P.C. 1976. [Flowering and sex expression in *Acer* L.] Mededelingen Lanbouwhogeschool Wageningen, Netherlands.

Dempsey, W.H. 1962. Pollen tube growth in vivo as a measure of pollen viability. Science 138: 436-437.

Dewers, R.S.; Moehring, D.M. 1970. Effect of soil water strees on initiation of ovulate primordia in loblolly pine. Forest Sci. 16: 219-221.

Dhawan, A.K.; Malik, C.P. 1981. Effect of growth regulators and light on pollen germination and pollen tube growth in *Pinus roxburghii* Sarg. Ann. Bot. 47: 239-248.

Dhir, K.K.; Chark, K.S.; Khurana, D.K.; Dua, I.S. 1982. Changes in the protein bands in pollen grains of *Populus ciliata* during storage and its effect on their viability and germination. Silvae Genet. 31: 6-8.

Diaz Luna, C.L. 1977. [Morphological anomalies in pollen grains of *Picea pinceana*.] Bull. Soc. Bot. Mexico 36: 3-11.

Dickinson, H.G. 1971. The role played by sporopollenin in the development of pollen in *Pinus banksiana*. Pages 31-67 *in* Brooks, J. et al., eds. Sporopollenin. Academic Press, Toronto.

Dickinson, H.G. 1976. Common factors in exine deposition. Pages 67-90 *in* Ferguson, I.K.; Muller, J., eds. The evolutionary significance of exine. Linnaean Soc., London.

Dickinson, H.G.; Bell, P.R. 1970. The development of the sacci during pollen formation in *Pinus banksiana*. Grana 10: 101-108.

Dickinson, H.G.; Bell, P.R. 1976. Development of the tapetum in *Pinus banksiana* preceding sporogenesis. Ann. Bot. 40: 103-113.

Dickmann, D.I.; Kozlowski, T.T. 1968. Mobilization by *Pinus resinosa* cones and shoots of ^{14}C photosynthate from needles of different ages. Am. J. Bot. 55: 900-906.

Dickmann, D.I.; Kozlowski, T.T. 1969a. Seasonal variations in reserve and structural components of *Pinus resinosa* cones. Am. J. Bot. 56: 515-520.

Dickmann, D.I.; Kozlowski, T.T. 1969b. Seasonal changes in the macro- and micro-nutrient composition of ovulate strobili and seeds of *Pinus resinosa*. Can. J. Bot. 47: 1547-1554.

Dickmann, D.I.; Kozlowski, T.T. 1970. Mobilization and incorporation of photo-assimilated ^{14}C by growing vegetative and reproductive tissues of adult *Pinus resinosa* Ait. trees. Plant Physiol. 45: 284-288.

Dillon, E.S.; Zobel, B.J. 1975. A simple test for viability of pine pollen. J. For. 55: 21-32.

Doak, C.C. 1935. Evolution of foliar types, dwarf shoots, and cone scales of *Pinus*. Ill. Biol. Monogr. 13: 1-106.

Dogra, P.D. 1964. Pollination mechanisms in gymnosperms. Pages 142-175 *in* Advances in Palynology. Ann. Rep. Nat. Bot. Gard., Lucknow., India.

Dogra, P.D. 1967. Seed sterility and disturbances in embryogeny in conifers with particular reference to seed testing and tree breeding in Pinaceae. Stud. For. Suec. 45: 97 p.

Dorman, K.W. 1976. The genetics and breeding of southern pines. USDA Forest Serv. Agric. Handbook 471.

Doyle, J. 1945. Developmental lines in pollination mechanisms in the Coniferales. Sci. Proc. Roy. Dublin Soc. 24: 43-62.

Doyle, J. 1954. Development in *Podocarpus nivalis* in relation to other podocarps. III. General conclusions. Sci. Proc. Roy. Dublin Soc. 26: 347-377.

Doyle, J. 1957. Aspects and problems of conifer embryology. Advan. Sci. London 14: 120-130.

Doyle, J.; Brennan, M. 1971. Cleavage pollyembryony in conifers and taxads - a survey. I. Podocarps, taxads, and taxodioids. Sci. Proc. Roy. Dublin Soc. 4A: 57-88.

Doyle, J.; O'Leary, M. 1935. Pollination in *Pinus*. Sci. Proc. Roy. Dublin Soc. 21: 181-190.

Duff, G.H.; Nolan, N.J. 1958. Growth and morphogenesis in the Canadian forest species. III. The time scale of morphogenesis at the stem apex of *Pinus resinosa* Ait. Can. J. Bot. 36: 687-706.

Duffield, J.W. 1954. Studies of extraction, storage, and testing of pine pollen. Silvae Genet. 3: 39-45.

Duffield, J.W.; Callaham, R.Z. 1959. Deep-freezing pine pollen. Silvae Genet. 8: 22-24.

Duhoux, E. 1972a. Evolution structural de la paroi du grain de pollen du *Juniperus communis* L. (Cupressacées), cultivé *in vitro*, au cours de la phase d'hydratation. C.R. Acad. Sc. Paris. Série D. 274: 2767-2770.

Duhoux, E. 1972b. Formation de la paroi du tube pollinque au cours de la germination du pollen chez le *Juniperus communis* L., cultivé in vitro. C.R. Acad. Sc. Paris. Série D. 274: 3238-3241.

Dunberg, A.R. 1976. Changes in gibberellin-like substances and indole-3-acetic acid in *Picea abies* during the period of shoot elongation. Physiol. Plant. 38: 186-190.

Dunberg, A.R. 1977. Juvenility, maturation, aging, and rejuvenation in woody plants. Pages 55-64 *in* Vegetative propagation of forest trees, Physiology and practice. Upssala, Sweden.

Dunberg, A.R. 1979. Flower induction in Norway spruce. Pages 139-157 *in* IUFRO Norway Spruce Meeting S.2.03.11 - S.2.02.11. Bucharest 1979.

Dunberg, A.R. 1980. Stimulation of flowering in *Picea abies* by gibberellins. Silvae Genet. 29: 51-53.

Dunberg, A.R.; Oden, P. 1983. Gibberellins and conifers. Pages 221-296 *in* Crozier, A., ed. The biochemistry and physiology of gibberellins. Vol. 2. Praeger, N.Y.

Durzan, D.J.; Campbell, R.A. 1979. Red light inhibits female cone production in white spruce trees. Pages 57-62 *in* Bonner, F., ed. Proc. Symp. Flowering and seed development in trees. IUFRO., 1978. Miss. State Univ., USDA Forest Serv. Southern Forest Exper. Stn.

Durzan, D.J.; Campbell, R.A.; Wilson, A. 1979. Inhibition of female cone production in white spruce by red light treatment during night under field conditions. Env. Exp. Bot. 19: 133-144.

Eames, A.J. 1913. The morphology of *Agathis australis*. Ann. Bot. 27: 1-36.

Ebell, L.F. 1962. Growth and cone production responses of Douglas-fir to chemical fertilization. Can. Dep. For. Pacific Forest Res. Centre. Rep. on Project B.C. 1, 1-41.

Ebell, L.F. 1967a. Physiology of flowering and cone production in Douglas-fir, 1965-66. Pages 33-34 *in* Proc. Tenth Mgt. Comm. For. Tree Breeding in Can. Part 2. Dep. For. Rural Devel. Can. Forestry Branch.

Ebell, L.F. 1967b. Cone production induced by drought in potted Douglas-fir. Can. Forest Serv., Bi-mon. Res. Notes 23: 26-27.

Ebell, L.F. 1970. Physiology and biochemistry of flowering of Douglas-fir. *In* Proc. Sexual Reproduction of Forest Trees. IUFRO Sect. 22., Working Group Mtg. Finland.

Ebell, L.F. 1971. Girdling: its effect on carbohydrate status and on reproductive bud and cone development of Douglas-fir. Can. J. Bot. 49: 453-466.

Ebell, L.F. 1972a. Cone induction response of Douglas-fir to form of nitrogen fertilizer and time of treatment. Can. J. Forest Res. 2: 317-326.

Ebell, L.F. 1972b. Cone production and stem growth response of Douglas-fir to rate and frequency of nitrogen fertilization. Can. J. Forest Res. 2: 327-338.

Ebell, L.F.; McMullan, E.E. 1970. Nitrogenous substances associated with differential cone production responses of Douglas-fir to ammonium and nitrate fertilization. Can. J. Bot. 48: 2169-2177.

Ebell, L.F.; Schmidt, R.L. 1964. Meteorological factors affecting conifer pollen dispersal on Vancouver Island. Can. Dep. For. Publ. 1036, 28 p.

Echols, R.M.; Mergen, F. 1956. Germination of slash pine (*P. elliottii*) pollen in vitro. Forest Sci. 2: 321-327.

Eckert, R.L.; Eckert, B.S. 1984. A procedure for the study of immunochemical properties of megagametophyte tissue of *Pinus strobus* L. Can. J. Forest Res. 14: 142-145.

Ehrenberg, C.E. 1960. Studies on the longevity of stored pine pollen. Medd. Stat. SkogsForskn-Inst. 49: 1-31.

Eis, S. 1967. Cone crops of white and black spruce are predictable. For. Chron. 43: 247-252.

Eis, S. 1973. Cone production of Douglas-fir and grand fir and its climatic requirements. Can. J. Forest Res. 3: 61-70.

Eis, S.; Craigdallie, D. 1981. Reproduction of conifers. A handbook for cone crops assessment. Environ. Can., Can. Forest Serv., Pacific Forest Res. Centre, Rep. BC-X-219. 9 p.

Ekberg, I.; Eriksson, G. 1967. Development and fertility of pollen in three species of *Larix*. Hereditas 57: 303-311.

Ekberg, I,; Eriksson, G.; Kartel, N.; Sulikova, Z. 1967. The meiotic development in male aspen. Stud. For. Suec. 58: 3-16.

Eldridge, K.G. 1966. A seed production experiment with *Pinus radiata*. Aust. For. 30: 43-50.

Enescu, V.; Giurgiu, V. 1968. Results in an experiment to stimulate seed production in *Pinus sylvestris*. Rev. Padurilor 83: 109-112.

Enescu, V.; Popescu, G.; Contescu, L. 1978. Blossoming artificially induced in a clonal orchard by *Pseudotsuga menziesii* (Mirb.) Franco. Page 1071 *in* Proc. THIRD World Consult. For. Tree Breeding. CSIRO Canberra Australia. 1977. FAO/IUFRO. Rep. FO-FTB-77-4/28:

Erickson, E.H.; Buchmann, S.L. 1983. Electrostatics and pollination. Pages 173-186 *in* Jones, C.E.; Little, R.J., eds. *Handbook of experimental pollination biology.* Scientific and Academic Editions, Toronto.

Eriksson, G. 1968a. Meiosis and pollen formation in *Larix*. Roy. Coll. For., Stockholm. 18 p.

Eriksson, G. 1968b. Temperature response of pollen mother cells in *Larix* and its importance for pollen formation. Stud. For. Suec. 63: 1-131.

Eriksson, G. 1970. Low-temperature induced irregularities in pollen mother cells of *Larix leptolepis*. Stud. For. Suec. 85. 14 p.

Eriksson, G.; Ekberg, I.; Ehrenberg, L.; Bevalacqua, B. 1966. Genetic changes induced by semiacute γ-irradiation of pollen mother cells in *Larix leptolepis* (Sieb. et Zucc.) Gord. Hereditas 55: 213-226.

Eriksson, G.; Ekberg, I.; Jonsson, A. 1970a. Meiotic investigations in pollen mother cells of Norway spruce cultivated in a plastic greenhouse. Hereditas 66: 1-20.

Eriksson, G.; Ekberg, I,; Jonsson, A. 1970b. Further studies on meiosis and pollen formation. Stud. For. Suec. 87: 1-65.

Eriksson, G.; Lindgren, K.; Werner, M. 1975. Granens blomningsbiologi ett hinder för en rationell skogstradsfordling? Sveriges Skogsliardsforb. Tidskr. 73: 413-426.

Esau, K. 1965. Plant Anatomy. 2nd Ed. John Wiley and Sons. New York. 767 p.

Evans, L.T., ed. 1969a. The induction of flowering. MacMillan and Co., Melbourne. 488 p.

Evans, L.T. 1969b. The nature of flower induction. Pages 457-480 *in* Evans, L.T., ed. The induction of flowering. MacMillan and Co., Melbourne.

Evans, L.T. 1971. Flower induction and the florigen concept. Ann. Rev. Plant Physiol. 22: 365-394.

Faegri, K.; Van Der Pijl, L. 1979. The principles of pollination ecology (3rd rev. ed.). Pergamon, Oxford. 248 p.

Farmer, R.E. 1964. Cottonwood flowering as related to cold requirements of flower buds. Forest Sci. 10: 296-299.

Farmer, R.E., Jr.; Pitcher, J.A. 1981. Pollen handling for Southern hardwoods. Pages 77-83 *in* Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv./Agric. Handbook 587.

Faulkner, R. 1966. A review of flower induction experiments and trials. 1948-63. British Forestry Comm. Ann. Rep. Forest Res. p. 207-219.

Favre-Duchartre, M. 1956. Contribution à l'étude de la reproduction chez *Ginkgo biloba*. Rev. Cytol. Biol. Végét. 17: 1-218.

Favre-Duchartre, M. 1958a. *Ginkgo*, an oviparous plant. Phytomorphology 8: 377-390.

Favre-Duchartre, M. 1962. A method of representation of vegetative biological cycles applied to *Ginkgo*, *Araucaria*, *Taxus*, *Cephalotaxus*, and *Ephedra*. Silvae Genet. 1: 1-28.

Fechner, G.H. 1979. The biology of flowering and fertilization. Pages 1-24 *in* Bonner, F., ed. Proc. Symp. Flowering and Seed Development in Trees. IUFRO. 1978. Miss. State Univ. USDA Forest Serv. Southern Forest Exper. Stn.

Felker, F.C.; Robitaille, H.A.; Hess, F.D. 1983. Morphological and ultrastructural development and starch accumulation during chilling of sour cherry buds. Am. J. Bot. 70: 376-386.

Ferguson, M.C. 1904. Contributions to the knowledge of the life history of *Pinus* with special reference to sporogenesis, the development of the gametophytes, and fertilization. Proc. Wash. Acad. Sci. 6: 1-153.

Fielding, J.M. 1960. Branching and flowering characteristics of Monterey pine. Aust. For. Timber Bur. Bull. 37. 59 p.

Florence, R.G. 1964. A comparative study of lowering and seed production in six blackbutt (*Eucalyptus pilularis* Sm.) forest stands. Aust. For. 28: 23-33.

Florence, R.G.; McWilliam, J.R. 1956. The influence of spacing on seed production. Z. Forstgenetik 5: 97-102.

Fober, H. 1976. Relation between climatic factors and Scots pine (*Pinus sylvestris* L.) cone crops in Poland. Arboretum Kornickie 21: 367-374.

Forbes, D.C. 1971. Loss of shortleaf pine flowers under natural conditions - a probable explantation for high losses in controlled pollinations. Tree Planters' Notes 22: 30.

Forbes, D.C. 1973. Problems and techniques associated with natural and controlled pollination of black cherry (*Prunus serotina* Ehrh.) Page 475 *in* Proc. 20th Northeast Forest Tree Improv. Conf.

Forshell, C.P. 1974. Seed development after self-pollination and cross-pollination of Scots pine, *Pinus sylvestris* L. Stud. For. Suec. 118: 1-36.

Foster, A.S.; Gifford, E.M., Jr. 1974. Comparative morphology of vascular plants. 2nd Ed. W.H. Freeman and Company, San Francisco. 751 p.

Foster, G.S.; Bridgwater, F. 1979. Viability tests to evaluate pollen reliability in loblolly pine controlled pollinations. Forest Sci. 25: 270-274.

Fowells, H.A. (compiler). 1965. Silvics of forest trees of the United States. USDA Forest Serv. Agric. Handbook No. 271. 762 p.

Francini, E. 1958. Ecologie comparata di *Pinus halepensis* Ill., *Pinus pinaster* Sol. e *Pinus pinea* L. sulla base del comportamento del gametofito femminile. Acad. Italiana Sci. For. 7: 107-172.

Franklin, E.C. 1971. Pollen management in southern seed orchards. Pages 218-223 *in* Proc. 11th Southern Forest Tree Imp. Conf. Atlanta, Ga.

Franklin, E.C. 1981. Genetic mutants as pollen markers. Pages 58-61 *in* Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv. Agric. Handbook No. 587.

Franklin, J.F.; Ritchie, G.A. 1970. Phenology of cone and shoot development of Noble fir and some associated true firs. Forest Sci. 16: 356-364.

Fraser, D.A. 1958. The relation of environmental factors to flowering in spruce. Pages 629-642 *in* Thimann, K.V., ed. The Physiology of Forest Trees. Ronald Press Co. N.Y.

Fraser, D.A. 1962. Apical and radial growth of white spruce (*Picea glauca* (Moench) Voss) at Chalk River, Ontario, Canada. Can. J. Bot. 40: 659-668.

Fraser, D.A. 1966. Vegetative and reproductive growth of black spruce (*Picea mariana* (Mill.) BSP.) at Chalk River, Ontario, Canada. Can. J. Bot. 44: 567-580.

Fraser, D.A. 1969. Photoperiod, thermoperiod and gibberellic acid and their effect on growth of several conifers. Pages 39-49 *in* Yeatman, C.W., ed. Proc. 11th Meeting Comm. Forest Tree Breeding 1968. Dep. Fish. For. Can. Forest Branch, Petawawa For. Exper. Stn.

Fraser, D.A. 1970. Interaction of gibberellic acid and photoperiod of reproductive and vegetative growth of white cedar seedlings (*Thuja occidentalis* L.) Pages 15-20 *in* Morgenstern, E.K. ed. Proc. 12th Meeting Comm. Forest Tree Breed. Dep. Fish. For. Can. Forest. Serv. Petawawa For. Exper. Stn.

Fujimoto, Y.; Watanabe, M.; Anma, Y. 1964. [The effects of gamma irradiation on the pollen germination of *Pinus densiflora* and *Pinus thunbergii*.] J. Jpn. For. Soc. 46: 389-394.

Fukuhara, N. 1961. Stimulation of flower buds differentiation in forest trees by gibberellin. IUFRO 13th Congress, Vienna. Part 2.

Fulford, R.M. 1960. The use of defoliating sprays for the control of biennial bearing in apple. J. Hort. Sci. 35: 202-213.

Furukoshi, T. 1978. Investigations on natural self-pollination in a seed orchard of *Cryptomeria japonica* D. Don. Pages 895-906 *in* Proc. Third World Consult. For. Tree Breeding. 1977. Vol. 2, CSIRO Canberra, Australia.

Gabriel, W.J. 1967. Reproductive behaviour in sugar maple: self-compatibility, cross-compatibility, agamospermy, and agamocarpy. Silvae Genet. 116: 165-168.

Germain, E.; Jalinat, J.; Marchou, M. 1973. Biologie florale du noyer (*Juglans régia* L.). Bull. Tech. Info. No. 282: 661-673. Station de Recherches d'Arboriculture Fruitiére, Pont-de-la-Maye, France.

Gianordoli, M. 1964. Contribution à l'étude du cycle de reproduction sexuée du Sciadopitys verticillata. C.R. Acad. Sci. (Paris). Série D. 258: 6203-6206.

Giertych, M.M. 1967. Analogy of the differences between male and female strobiles in *Pinus* to the differences between long- and short-day plants. Can. J. Bot. 45: 1907-1910.

Giertych, M.M. 1970. The influecne of defoliation on flowering in pine, *Pinus sylvestris*. Arboretum Kornickie 15: 93-98.

Ciertych, M.M. 1973. Relation between nutrition, growth, and flowering in coniferous trees. Polish Acad. Sci. Instit. Dendrol. and Kornik Arbor., Third Ann. Report 1973.

Gifford, E.M., Jr.; Mirov, N.T. 1960. Initiation and ontogeny of the ovulate strobilus in Ponderosa pine. Forest Sci. 6: 19-25.

Goddard, R.E.; Matthews, F.R. 1981. Pollen testing. Pages 40-43 *in* Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv. Agric. Handbook 587.

Godman, A.M. 1962. Red pine cone production stimulated by heavy thinning. USDA Forest Serv. LSFES Tech. Note 628. 2 p.

Graf, J. 1921. Beiträge zur Kenntnis der Gattung *Populus*. Beitr. Bot. Cent. 38. Abt. 1: *405-454*.

Grainger, J. 1938. Studies upon the time of flowering of plants. I. The relation of nocturnal translocation to the time of flowering. Ann. Appl. Biol. 25: 1-19.

Grano, C.X. 1973. Loblolly pine fecundity in South Arkansas. USDA Forest Serv. Res. Note No. SO-159. 7 p.

Graves, A.H. 1958. Hastening flowering in a chestnut seedling. Rep. Northern Nut Growers Assn. No. 49: 90-94.

Greenwood, M.S. 1977. Seed orchard fertilization: optimizing time and rate of ammonium nitrate application for grafted loblolly pine (*Pinus taeda* L.). *In* Proc., Southern Tree Imp. Conf., Gainesville, Fla. 14: 164-169.

Greenwood, M.S. 1978a. Flowering induced on young loblolly pine grafts by out-of-phase dormancy. Science 201: 443-444.

Greenwood, M.S. 1978b. Flower stimulation techniques for loblolly pine (*Pinus taeda* L.). Pages 1031-1042 *in* Proc. Third World Consult. For. Tree Breeding. 1977, Vol. 2. CSIRO, Canberra, Australia.

Greenwood, M.S. 1980. Reproductive development in loblolly pine. I. The early development of male and female strobili in relation to the long shoot growth behaviour. Am. J. Bot. 67: 1414-1422.

Greenwood, M.S. 1981. Reproductive development in loblolly pine. II. The effect of age, gibberellin plus water stress and out-of-phase dormancy on long shoot growth behaviour. Am. J. Bot. 68: 1184-1190.

Greenwood, M.S. 1982. Rate, timing, and mode of gibberellin application for female strobilus production by grafted loblolly pine. Can. J. Forest Res. 12: 998-1002.

Greenwood, M.S.; Gladstone, W.T. 1978. Topworking loblolly pine for precocious flowering. For. Res. Tech. Rep. 042-3004/78/80, Weyerhauser Corp. 8 p.

Gregory, J.D.; Davey, C.B. 1977. Subsoiling to stimulate flowering and cone production in a loblolly pine seed orchard. Southern J. Appl. For. 1: 20-23.

Grisez, T.J. 1975. Flowering and seed production in seven hardwood species. USDA Forest Serv. Res. Paper No. NE-315. 8 p.

Guignard, J.L.; Mestre, J.C. 1966. Sur le développement d'embryons à partir des antipodes chez l'*Ulmus campestris* L. Soc. Bot. Fr. Bull. *113: 227-228.*

Hadders, G. 1977. Experiments with supplemental mass pollination in seed orchards of Scots pine (*Pinus sylvestris* L.). *In* Proc. Third World Consult. of For. Tree Breeding. CSIRO, Canberra, Australia. Report FO-FTB-77-4/19. 12 p.

Hagem, O. 1917. Furuens og granens frosaetningi Norge. Medd. Vestlandets Forstl. Fors. Stat. 1:2:1-188. Cited in: Andersson, E. 1965. Cone and seed studies in Norway spruce (*Picea abies* (L.) Karst). Stud. For. *Suec. 23*: 1-214.

Hagman, M. 1970. Observations on incompatibility in *Alnus*. Pages 1-19 *in* Proc. *Sexual reproduction in Forest* trees. IUFRO Sect. 22. Working Group Mtg. Finland.

Hagman, M. 1971. On self- and cross-incompatibility shown by *Betula verracosa* Ehrh. and *Betula pubescens* Ehrh. Commun. Inst. Forest. Fenn. 73.6: 1-125.

Hagman, M. 1975. Incompatibility in forest trees. Proc. Roy. Soc. Lond. B. 188: 313-326.

Hagman, M.; Mikkola, L. 1963. Observations on cross-, self-, and interspecific pollinations in *Pinus peuce* Griseb. Silvae Genet. 12: *73-79.*

Hakansson, A. 1956. [Seed development of *Picea abies* and *Pinus sylvestris*.] Medd. Statens Skogforskningsinst. 46: 1-23.

Halevy, A.H., ed. 1985. Handbook of flowering. Vols. I, IV. CRC Press, Boca Raton, Fla.

Hall, J.P. 1977. Effect of exogenous growth substances on flowering grafted cones of *Larix*. Dep. Envir. Can. Can. Forest Serv., Nfld. Forest Res. Centre. Rep. N-X-151. 11 p.

Hall, J.P. 1982. Microsporogenesis in *Larix laricina*. Can. J. Bot. 60: 797-805.

Halls, L.K.; Hawley, N.R. 1954. Slash pine cone production is increased by seed-tree release. USDA Forest Serv. Southeast Forest Exper. Stn. Res. Note. No. 66. 2 p.

Hansbrough, T.; Merrifield, R.G. 1963. The influence of partial girdling on cone and seed production of loblolly pine. Louisiana St. Univ., For. Note No. 52. 2 p.

Hard, J.S. 1963. Frost damage to red pine conelets. USDA Forest Serv. Lake States Forest Exper. Stn., Res. Note. No. LS-5.

Hare, R.C. 1979. Promoting flowering in loblolly and slash pine with branch, bud, and fertilizer treatments. Pages 112-121 *in* Bonner, F., ed. Proc. Symp. Flowering and seed development in trees. IUFRO, 1978. Miss. State Univ. USDA Forest Serv. Southern Forest Exper. Stn.

Hare, R.C. 1984. Application method and timing of gibberellin $A_{4/7}$ treatments for increased pollen conebud production in southern pines. Can. J. Bot. 14: 128-131.

Hare, R.C.; Snyder, E.B.; Schmidtling, R.C. 1979. Longleaf pine flowering in response to nitrogen fertilization, branch girdling, growth substances, and cultivation. Pages 11-16 *in* Proc. 13th Lake States For. Tree Improv. Conf. 1977. Univ. Minn., St. Paul. (Also published as USDA Forest Serv. Gen. Tech. Rep. NC-50.

Harrison, D.L.S.; Owens, J.N. 1983. Bud development in *Picea engelmannii*. I. Vegetative bud development, differentiation, and early development of reproductive buds. Can. J. Bot. 61: 2291-2301.

Hashizume, H. 1959. [The effect of gibberellin upon flower formation and sex transition to female in *Chamaecyparis obtusa* and *C. lawsoniana*.] J. Jpn. For. Soc. 41: 158-161.

Hashizume, H. 1961. [The effect of gibberellin on sex differentiation in *Cryptomeria japonica* strobiles. II. Effects of auxin and urea on gibberellin-induced sex transition to female in strobiles.] J. Jpn. For. Soc. 43: 47-49.

Hashizume, H. 1967. [Experimental induction of female flowers in young Japanese larch (*Larix leptolepis* Gordon).] J. Jpn. For. Soc. 49: 405-408.

Hashizume, H. 1969. [Auxins and gibberellin-like substances existing in the shoots of conifers and their roles in flower bud formation and flower sex differentiation.] Tottori Univ. For. Jpn. Bull. 4. 46 p.

Hashizume, H. 1970. [Effect of mechanical treatments on flower bud formation in conifers and change of nutrients in shoots in relation to flower induction.] Fac. Agric. Tottori Univ. Jpn. Bull. 22: 25-33.

Hashizume, H. 1973. [Studies on flower bud formation, flower sex differentiation and their control in conifers.] Tottori Univ. Jpn. For. Bull. 7: 1-139.

Hashizume, H. 1975. [Interrelation of gibberellin and ethrel in the flower bud formation and growth of conifers.] Tottori Univ. Jpn. For. Bull. 8: 1-10.

Hattemer, H.H.; Andersson, E.; Tamm, C.O. 1977. Effects of spacing and fertilization on four grafted clones of Scots pine. Stud. For. Suec. 141: 31 p.

Hauser, E.J.P.; Morrison, J.H. 1964. The cytochemical reduction of nitro blue tetrazolium as an index of pollen viability. Am. J. Bot. 51: 748-752.

Heidmann, L.J.; Rietveld, W.J.; Trujillo, D.P. 1979. Fertilization increases cone production and diameter growth of a

55-year old Ponderosa pine stand in Arizona. Pages 197-205 *in* Bonner, F., ed. Proc. Symp. Flowering and seed development in trees. IUFRO, 1978. Miss. State Univ. USDA Forest Serv. Southern Forest Exper. Stn.

Heitmuller, H.H.; Melchior, G.H. 1960. Über die blühfördernde Wrikung des Wurzelschnitts, des Zweigkrümmens und der Strangulation au japanischer Lärche (*Larix leptolepis* [Sieb. et Zucc.] Gord.). Silvae Genet. 9: 65-72.

Heslop-Harrison, J. 1971. Pollen: development and physiology. Butterworth, London. 338 p.

Heslop-Harrison, J. 1975. The physiology of the pollen grain surface. Proc. Roy. Soc. Lond. B. 190: 275-299.

Heslop-Harrison, J. 1976. A new look at pollination. Pages 141-157 *in* Rep. E. Malling Res. Stn. for 1975. East Malling, Kent, England.

Heslop-Harrison, J. 1978. Recognition and response in the pollen-stigma interaction. *In* Curtis, A.S.G., ed. Cell-Cell Recognition Symp. Soc-exp. Biol. 32: 121-138. Cambridge Univ. Press.

Heslop-Harrison, J.; Heslop-Harrison, Y. 1970. Evaluation of pollen viability by enzymatically induced fluorescence: intracellular hydrolysis of fluorescein diacetate. Stain Technology 45: 115-120.

Heslop-Harrison, J.; Heslop-Harrison, Y. 1982. The growth of the grass pollen tube: I. Characteristics of the polysaccharide particles (P-particles) associated with apical growth. Protoplasma 112: 71-80.

Heslop-Harrison, J.; Heslop-Harrison, Y.; Barber, J. 1975. The stigma surface in incompatibility responses. Proc. Roy. Soc. Lond. B. 188: 287-297.

Heslop-Harrison, J.; Heslop-Harrison, Y.; Shivanna, K.R. 1984. The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure. Theor. Appl. Genet. 67: 367-3754.

Heslop-Harrison, Y. 1977. The pollen-stigma interaction: pollen-tube penetration in *Crocus*. Ann. Bot. 41: 913-922.

Heslop-Harrison, Y.; Shivanna, K.R. 1977. The receptive surface of the angiosperm stigma. Ann. Bot. 41: 1233-1258.

Hibbs, D.E.; Fischer, B.C. 1979. Sexual and vegetative reproduction of striped maple (*Acer pensylvanicum* L.). Bull. Torr. Bot. Club. 106: 222-227.

Hillman, W.S. 1962. The physiology of flowering. Holt, Rinehart, and Winston, Toronto. 164 p.

Hillman, W.S. 1964. Endogenous circadian rythms and the response of *Lemna perpusilla* to skeleton photoperiods. *In* Symposium on time measurement in photoperiodic phenomena, Cleveland, Ohio. 1963. Am. Nat. 98: 323-328.

Hjelmqvist, H. 1953. The embryo sac development of *Quercus robur* L. Phytomorphology 3: 377-384.

Ho, R.H. 1980. Pollination mechanism and seed production potential in Douglas-fir. Forest Sci. 26: 522-528.

Ho, R.H. 1982. Research on seed-cone receptivity, seed production potential, cone induction, and haploid plant culture. Pages 116-118 *in* Yeatman, C.W., ed. Proc. 18th Meeting Can. Tree Improv. Assoc. 1981. Environ. Can., Can. For. Serv., Petawawa Natl. For. Inst.

Ho, R.H.; Owens, J.N. 1974a. Microstrobilate morphology, microsporogenesis and pollen formation in western hemlock. Can. J. Forest Res. 4: 509-517.

Ho, R.H.; Owens, J.N. 1974b. Microstrobili of Douglas-fir. Can. J. Forest Res. 4: 561-562.

Ho, R.H.; Owens, J.N. 1974c. Microsporogenesis and pollen formation in lodgepole pine. Can. J. Bot. 52: 1669-1674.

Ho, R.H.; Rouse, G.E. 1970. Pollen germination of *Larix sibirica* (Siberian larch) in vitro. Can. J. Bot. 48: 213-215.

Ho, R.H.; Sziklai, O. 1971a. Germination and development of lodgepole pine pollen in vitro. Can. J. Forest Res. 1: 12-19.

Ho, R.H.; Sziklai, O. 1971b. Pollen germination of *Tsuga heterophylla* in vitro. Can. J. Bot. 49: 117-119.

Ho, R.H.; Sziklai, O. 1972. Germination of Douglas-fir pollen. Silvae Genet. 21: 48-50.

Hocker, H.W., Jr. 1962. Stimulating conelet production of eastern white pine. Pages 35-40 *in* Proc. Ninth Northeastern For. Tree Imp. Conf.

Hodson, H.K.; Hamner, K.C. 1970. Floral inducing extracts from *Xanthium*. Science 167: 384-385.

Hoekstra, P.E.; Mergen, F. 1957. Experimental induction of female flowers on young slash pine. J. For. 55: 827-831.

Holmes, G.D.; Matthews, J.D. 1951. Girdling or banding as a means of increasing cone production in pine plantations. British Forestry Comm. For. Res. No. 12: 1-8.

Holmsgaard, E. 1972. Relations between climate and flowering, seed production and growth. Forest Tree Imp. 4: 53-66.

Holmsgaard, E.; Olson, H.C. 1960. The influence of weather on beech mast. Forstl. Forogsv. Danm: 26: 345-370.

Holmsgaard, E.; Olson, H.C. 1961. On the influence of the weather on beech mast and the employment of artificial drought as a means to produce beech mast. *In* IUFRO 13th Cong. Vienna Pt. 2(1).

Holmsgaard, E.; Olson, H.C. 1966. Experimental induction of flowering in beech. Forstl. Forogsv. Danm. 30: 2-17.

Holst, M.J. 1959. Experiments with flower promotion in *Picea glauca* (Moench) Voss and *Pinus resinosa* Ait. *In* Recent advances in Botany, Toronto Univ. Press. Vol. 2: 1654-1658.

Holst, M.J. 1971. The stimulation of flowering in spruce and pine. Pages 125-128 *in* Morgenstern, E.K., ed. Proc. 12th Meeting Comm. For. Tree Breeding 1970. Dep. Fish. For. Can. For. Serv., Petawawa Forest Exper. Stn.

Hutchinson, A.H. 1915. On the male gametophyte of *Picea canadensis*. Bot. Gaz. 59: 287-300.

Hutchinson, J.G.; Bramlett, D.L. 1964. Frost damage to shortleaf pine flowers. J. For. 62: 343.

Ichikawa, S.; Shidei, T. 1971. [Fundamental studies on deep-freezing storage of tree pollen.] Kyoto Univ. Jpn. For. Bull. 42: 51-82.

Ichikawa, S.; Shidei, T. 1972a. [Fundamental studies of deep-freezing on tree pollen (II).] Kyoto Univ. Jpn. For. Bull. 43: 9-32.

Ichikawa, S.; Shidei, T. 1972b. [Fundamental studies on deep-freezing storage of tree pollen (III).] Kyoto Univ. Jpn. For. Bull. 44: 47-67.

Ichikawa, S,; Kaji, K.; Kubota, Y. 1970. [Studies on the storage of larch (*Larix leptolepis*) pollen at super-low temperatures. Some problems concerning water contents and pre-freezing (I).] Hokkaido Forest Exper. Stn. Jpn. Bull. 8: 11-26.

Illy, G. 1963. Relation entre la hauteur des arbres et la fructification du pine maritime (*Pinus pinaster* Sol.) *In* World Consult. on For. Genet. and For. Tree Imp. Stockholm 1963. FAO/Forgen. 63 8/7.

Jackson, D.I.; Sweet, G.B. 1972. Flower initiation in temperate woody plants. A review based largely on the literature of conifers and deciduous fruit trees. Horticult. Abstr. 42: 9-24.

Janas, P.S.; Haddon, B.D. 1984. Canadian forest tree seed statistics: 1980-81 Survey Results. Can. For. Serv., Petawawa Natl. For. Inst. Rep. PI-X-41. 23 p.

Jensen, C.J. 1964. Pollen storage under vacuum. Danish Atomic Energy Comm. Kongelige Veterinarerog Landbohjskole Arsskrift. p. 133-146.

Johansen, D.A. 1950. Plant embryology. Chronica Botanica Co., Waltham, Mass. 305 p.

Johnson-Flanagan, A.M.; Owens, J.N. 1985. Development of white spruce (*Picea glauca* (Moench) Voss) seedling roots. Can. J. Bot. 63: 456-462.

Johnstone, G.R. 1940. Further studies on polyembryony and germination of polyembryonic pine seeds. Am. J. Bot. 46: 425-433.

Johri, B.M. 1963. The Female gametophyte. Pages 69-104 *in* Maheshwari, P., ed. Recent advances in the embryology of Angiosperms. Catholic Press, Ranchi, India.

Johri, B.M.; Vasil, I.K. 1961. Physiology of pollen. Bot. Rev. 27: 325-381.

Jones, C.E.; Little, R.J. eds. 1983. Handbook of experimental pollination biology. Scientific and Academic Editions, Toronto. 558 p.

Jonsson, A. 1974. A study on the temperature response of pollen mother cells in Norway spruce. Stud. For. Suec. 116: 1-32.

Jovancevic, M. 1962. Estimation of tree pollen germination from the size, shape, and color of the grains. Nar. Sumar, Sarajevo 16: 493-502.

Junttila, O. 1980. Flower bud differentiation in *Salix pentandra* as affected by photoperiod, temperature, and growth regulators. Physiol. Plant. 49: 127-134.

Kamienska, A.; Pharis, R.P. 1975. Endogenous gibberellins of pine pollen. II. Changes during germination of *Pinus attenuata*, *P. coulteri*, and *P. ponderosa* pollen. Plant Physiol. 56: 655-659.

Kamienska, A.; Durley, R.C.; Pharis, R.P. 1976a. Isolation of gibberellin A_3, A_4, and A_7 from *Pinus attenuata* pollen. Phytochemistry 15: 421-424.

Kamienska, A.; Durley, R.C.; Pharis, R.P. 1976b. Endogenous gibberellins of pine pollen. III. Conversion of 1, 2-[^{3}H] GA_4 to gibberellins A_1 and A_3, in germinating pollen of *Pinus attenuata*. Lemn. Plant Physiol. 58: 68-70.

Kanekawa, T.; Katsuta, M. 1982. [Promotion of strobilis production in *Pinus thunbergii* Parl. and *P. densiflora* Sieb. et Zucc. by gibberellins.] J. Jpn. For. Soc. 64: 101-106.

Kapil, R.N.; Bhatnagar, A.K. 1975. A fresh look at the process of double fertilization in Angiosperms. Phytomorphology 25: 334-368.

Karnosky, D.F.; Stairs, G.R. 1974. The effects of SO_2 on in vitro forest tree pollen germination and tube elongation. J. Environ. Quality 3: 406-409.

Kashimura, D.; Saito, H.; Kida, S. 1953. [A research on the Buna (*Fagus crenata* Blume) coppice forest. II. On sprouting condition after cutting (2)]. J. Jpn. For. Soc. 35: 154-156.

Kato, Y,; Fukuhara, N.; Kobayashi, R. 1959. Stimulation of flower bud differentiation of conifers by gibberellins. J. Jpn. For. Soc. 41: 309-311.

Katsumata, T,; Takahashi, N.; Ejiri, S. 1978. Changes of cyclic AMP level and adenylate cyclase activity during germination of pine pollen. Agric. Biol. Chem. 42: 2161-2162.

Katsuta, M. 1975. Effects of chilling and photoperiodic treatments on cone development in *Pinus thunbergii* Parl. and *P. densiflora* Sieb. et Zucc. Tokyo Univ. Jpn. For. Bull. 67: 63-104.

Katsuta, M. 1981. Recent advances in the promotion of strobilus production by gibberellins in Japanese conifers. Pages 68-74 *in* Krugman, S.; Katsuta, M., eds. Proc. Symp. on Flowering Physiol. 17th IUFRO World Congr., Kyoto, Japan. Jpn. For. Tree Breeding Assoc., Tokyo.

Katsuta, M.; Satoo, T. 1964. [Cone development in *Pinus thunbergii*.] J. Jpn. For. Soc. 46: 166-170.

Katsuta, M.; Saito, M.; Yamamoto, C.; Kaneko, T.; Itoo, M. 1981. [Effect of gibberellins on the promotion of strobilus production in *Larix leptolepis* Gord. and *Abies homolepis* Sieb. et

Zucc.] For. and For. Prod. Res. Inst. Jpn. Bull. 313: 37-45.

Kessler, K.J., Jr. 1969. Top-dying of yellow birch associated with seed production. Plant Disease Reporter 53: 694-697.

King, J.R. 1965. The storage of pollen---particularly by the freeze-drying method. Bull. Torrey Bot. Club 92: 270-287.

Klaehn, F.U. 1961. Flower morphology and flower ecology with respect to forest tree breeding procedures. *In* Recent Advances in Botany. Univ. Toronto Press. Vol. 2: 1666-1671.

Klebs, G. 1910. Alterations in the development and forms of plants as a result of environment. Proc. Roy. Soc. Lond. B. 82: 547-558.

Klebs, G. 1918. Uber die Blütenbildung bei Sempervirum. Flora 11-12: 128-151.

Knox, R.B.; Willing, R.R.; Ashford, A.E. 1972a. Role of pollen-wall proteins as recognition substances in interspecific incompatibility in poplars. Nature 237: 381-383.

Knox, R.B.; Willing, R.R.; Pryor, L.D. 1972b. Interspecific hybridization in poplars using recognition pollen. Silvae Genet. 21: 65-69.

Kolpak, M.X.; Feret, P.P.; Stipes, R.J. 1980. Fungi associated with the pistillate flowers of white oak and their effect on pollen germination. For. Ecol. Manag. 3: 69-72.

Konar, R.N.; Oberoi, Y.B. 1969a. Recent work on reproductive structures of living conifers and taxads - A review. Bot. Rev. 35: 89-116.

Konar, R.N.; Oberoi, Y.B. 1969b. Studies on the morphology and embryology of of *Podocarpus gracilor*. Pilger. Bietr. Biol. Pflanzen. 45: 329-376.

Kormutak, A. 1984. Some cytological and biochemical aspects of interspecific incompatibility in pines (*Pinus* sp.). Acta Dendrobiologica 7: 1-92.

Kossuth, S.U.; Fechner, G.H. 1973. Incompatibility between *Picea pungens* Engelm. and *Picea engelmannii* Parry. Forest Sci. 19: 50-60.

Kotter, W. 1931. Normale and anomale Fruchtbildung bei *Juniperus communis*. Inagural Diss. Univ. Hamburg, pp. 1-75. Cited in Singh, H. 1973. Embryology of Gymnosperms. Gebrüder Brontraeger Berlin. p. 248.

Kozlowski, T.T. 1971. Growth and development of trees. Vol. I. Seed germination, ontogeny, and shoot growth. Academic Press, New York. 443 p.

Kozlowski, T.T.; Keller, T. 1966. Food relations of woody plants. Bot. Rev. 32: 293-382.

Kramer, P.J.; Kozlowski, T.T. 1960. Physiology of trees. McGraw-Hill Co. N.Y. 642 p.

Kraus, E.J. 1925. Soil nutrients in relation to vegetation and reproduction. Am. J. Bot. 12: 510-516.

Kriebel, H.B. 1966. Technique and interpretation in tree seed radiography. Pages 70-75 *in* Joint Proc. 2nd Gen. Workshop Soc. Am. Foresters and the Seventh Lake States For. Tree Impr. Conf. 1965. USDA Forest Serv. Res. Paper No. 6.

Krugman, S.L. 1966. Freezing spring temperatures damage knobcone pine conelets. USDA Forest Serv. Res. Paper No. PSW-37.

Krugman, S.L. 1970. Incompatibility and inviability systems among some western North American pines. *In* Proc. Sexual Reproduction of Forest Trees. IUFRO Sect. 22, Working Group Mtg. Finland. 12 p.

Krugman, S.L.; Stein, W.I.; Schmitt, D.M. 1974. Seed Biology. Pages 1-40 *in* Schopmeyer, C.S., ed. Seeds of woody plants in the United States. USDA Forest Serv., Agric. Handbook 450.

Krusche, D.; Melchior, G.H. 1978. The choice of rootstock as a means to stimulate flowering and increase the cone yield in Norway spruce grafts. Pages 1079-1089 *in* Proc. Third World

Consult For. Tree Breeding, 1977. Vol. 2: CSIRO, Canberra, Australia.

Kudo, H. 1980. [Development of male flower and germination of pollen in 11 species of Pinaceae.] Hokkaido Univ. Jpn. Coll. Exp. For. Research Bull. 37: 143-164.

Kuhlwein, H.; Anhaeusser, H. 1951. Verӓnderungen des Gymnospermen pollen durch Lagerung. Planta 39: 476-479.

Kupila-Ahvenniemi, S.; Pihakaski, S.; Pihakaski, K. 1978. Wintertime changes in the ultrastructure and metabolism of the microsporangiate strobili of Scotch pine. Planta 144: 19-29.

La Bastide, J.G.A.; Van Vredenburch, C.L.H. 1970. The influence of weather conditions on the seed production of some forest trees in the Netherlands. Meded. Bosbouwproefstation No. 102: 1-12.

Lang, A. 1956. Gibberellin and flower formation. Naturwissenschaften 43: 544.

Lang, A. 1957. The effect of gibberellin upon flower formation. Proc. Nat. Acad. Sci. 43: 709-717.

Lanner, R.M. 1963. Growth and cone production of Knobcone pine under interrupted nights. USDA Forest Serv. Res. Paper No. PSW-38. 16 p.

Lanner, R.M.; Van Den Berg, D.A. 1975. The vegetative buds and shoots of lodgepole pine. Pages 68-85 *in* Proc. Management of lodegepole pine ecosystems. Vol. 1. Pullman, Wash.

Larson, D.A. 1965. Fine-structural changes in the cytoplasm of germinating pollen. Am. J. Bot. 52: 139-154.

Lavender, D.P. 1985. Angiospermous forest trees of temperate zones. Pages 15-22 *in* Halevy, A.H., ed. Handbook of flowering. CRC Press Inc. Boca Raton, Florida. Vol. I.

Lavender, D.P.; Zaerr, J.B. 1985. Temperate conifer forest trees. Pages 1-13 *in* Halevy, A.H., ed. Handbook of flowering. CRC Press Inc. Boca Raton, Florida. Vol. I.

Lawrence, G.H.M. 1951. Taxonomy of vascular plants. MacMillan Co. Can. Ltd. Toronto. 823 p.

Lawson, A.A. 1910. The gametophytes and embryo of *Sciadopitys verticillata*. Cytologia: Fujii Jubilee Vol. 14: 19.

Ledig, F.T.; Beland, J.W., IV; Fryer, J.H. 1971. Breeding techniques for white oak. Pages 275-283 *in* Proc. Eleventh Southern Conf. For. Tree Imp.

Lee, K.J. 1979. Factors affecting cone initiation in Pines: a review. Korean Inst. of For. Genet. Res. Rep. No. 15: 45-85.

Leopold, A.C. 1964. Plant growth and development. McGraw Hill, New York. 466 p.

Leopold, A.C.; Kriedemann, P.E. 1975. Plant growth and development. McGraw-Hill Inc., Toronto. 545 p.

Lepisto, M. 1973. Accelerated birch breeding in plastic greenhouses. For. Chron. 49: 172-173.

Leshem, Y.; Ophir, D. 1977. Differences in endogenous levels of gibberellin activity in male and female partners of two dioecious tree species. Ann. Bot. 41: 375-379.

Lester, D.T. 1963. Floral initiation and development in quaking aspen. Forest Sci. 9: 323-329.

LeTacon, F.; Oswald, H.; Bitsch, M.; Bouchard, D.; Divoux, A. 1977. Influence de la fertilization minérale sur la fructification du Hetre (*Fagus silvatica*). Ann. Sci. Forest. 34: 89-109.

Lewis, R.A. 1981. Biochemical gene markers. Pages 58-61 *in* Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv. Agric. Handbook No. 587.

Li, S.J. 1975. Reproductive biology of *Chamaecyparis*. II. Pollen development and pollination mechanism. Taiwania 20: 139-146.

Li, W.Y. 1964. The incompatibility resulting from the hybridization between

Pinus koraiensis and Pinus tabulaeformis. Scientia Silvae 9: 279-296.

Lill, B.S. 1974. Development of the female cone of Pinus radiata D. Don. Vol. I. Ph.D. Thesis, Univ. Canterbury, Christchurch, N.Z.

Lill, B.S.; Sweet, G.B. 1977. Pollination in Pinus radiata. N.Z.J. For. Sci. 7: 21-34.

Lindgren, K.; Edberg, I.; Eriksson, G. 1977. External factors influencing female flowering in Picea abies (L.) Karst. Stud. For. Suec. 142. 53 p.

Linnaeus, C. 1751. Skanska Resa. Stockholm. Cited in Holmsgaard, E. 1972. Relations between climate and flowering, seed production, and growth. For. Tree Imp. 4: 53-66.

Linskens, H.F. 1964. Pollen physiology and fertilization. North Holland Publishing Co., Amsterdam. 257 p.

Linskens, H.R. ed. 1974. Fertilization in higher plants. American Elsevier, New York. 373 p.

Livingston, G.K. 1971a. The morphology and behaviour of meiotic chromosomes of Douglas-fir. Silvae Genet. 20: 75-82.

Livingston, G.K. 1971b. Experimental studies on the induction of haploid parthenogenesis in Douglas-fir and the effects of radiation on the gemrination and growth of Douglas-fir pollen. Ph.D. Thesis, Univ. of Washington.

Livingston, G.K.; Ching, K.K. 1967. The longevity and fertility of freeze-dried Douglas-fir pollen. Silvae Genet. 16: 98-101.

Livingstin, G.K.; Stettler, R.F. 1973. Radiation induced stimulation of pollen-tube elongation in Douglas-fir. Rad. Bot. 13: 65-72.

Loach, K.; Little, C.H.A. 1973. Production, storage, and use of photosynthate during shoot elongation in balsam fir (Abies balsamea). Can. J. Bot. 51: 1161-1168.

Lohwag, H. 1910. Beitrag zur kenntnis der Zeit ersten Blütenanlage bei Holzpflanzen. Osterr. Bot. Z. 60: 369-376.

Longman, K.A. 1961. Factors affecting flower initiation in certain conifers. Proc. Linn. Soc. Lond. 172: 124-127.

Longman, K.A. 1976. Some experimental approaches to the problem of phase change in forest trees. Acta Hort. 56: 81-90.

Longman, K.A. 1982. Effects of gibberellin, clone, and environment on cone initiation, shoot growth and branching in Pinus contorta. Ann. Bot. 50: 247-257.

Longman, K.A.; Wareing, P.F. 1958. Effect of gravity on flowering and shoot growth in Japanese larch (Larix leptolepis Murray). Nature 182: 380-381.

Longman, K.A.; Wareing, P.F. 1959. Early induction of flowering in birch seedlings. Nature 184: 2037-2038.

Longman, K.A.; Nasr, T.A.A.; Wareing, P.F. 1965. Gravimorphism in trees. 4. The effect of gravity on flowering. Ann. Bot. 29: 459-472.

Looby, W.J.; Doyle, J. 1937. Fertilization and proembryo formation in Sequoia. Sci. Proc. Roy. Dublin Soc. 21: 457-476.

Looby, W.J.; Doyle, J. 1939. The ovule, gametophyte, and proembryo in Saxegothaea. Sci. Proc. Roy. Dublin Soc. 22: 95-117.

Looby, W.J.; Doyle, J. 1942. Formation of gynospore, female gametophyte, and archegonia in Sequoia. Sci. Proc. Roy. Dublin Soc. 23: 35-54.

Looby, W.J.; Doyle, J. 1944a. The gametophyte of Podocarpus andinus. Sci. Proc. Roy. Dublin Soc. 23: 222-237.

Looby, W.J.; Doyle, J. 1944b. Fertilization and early embryogeny in Podocarpus andinus. Sci. Proc. Roy. Dublin Soc. 23: 257-270.

Lorenzi, R.; Horgan, R.; Wareing, P.F. 1976. Endogenous hormones of Sitka spruce. British For. Comm., Ann. For. Res. Rep. 1976: 60.

Lowry, W.P. 1966. Apparent meteorological requirements for abundant cone crop in Douglas-fir. Forest Sci. 12: 185-192.

Luckwill, L.C. 1959. Fruit growth in relation to internal and external chemical studies. Pages 233-251 *in* Rudnick, R., ed. Cell, organism, and milieu. Ronald Press, New York.

Luckwill, L.C. 1970. The control of growth and fruitfulness of apple trees. Pages 237-253 *in* Cutting, C.V.; Luckwill, L.C., eds. Physiology of crop trees. Academic Press, New York.

Lunden, R. 1954. A short introduction to the literature on pollen chemistry. Svensk. Kem. Tidskr. 66: 201-213.

Luomajoki, A. 1977. Effects of temperature on spermatophyte male meiosis. Hereditas 85: 33-48.

Luomajoki, A. 1982. Temperature and dates of male meiosis in trees. Hereditas 97: 167-178.

Luukkanen, O. 1979. Hormonal treatment increases flowering of Norway spruce grafts in a plastic greenhouse. Finnish Found. For. Tree Breeding Ann. Rep. p. 20-26.

Luukkanen, O. 1981. Effects of gibberellins GA_4 and GA_7 on flowering in Scots pine grafts. Silvae Fenn. 15: 359-365.

Luukkanen, O.; Johansson, S. 1980a. Flower induction by exogenous plant hormones in Scots pine and Norway spruce grafts. Silvae Fenn. 14: 95-105.

Luukkanen, O.; Johansson, S. 1980b. Effect of exogenous gibberellins of flowering in *Pinus sylvestris* grafts. Physiol. Plant. 50: 365-370.

Lyons, L.A. 1956. The seed production capacity and efficiency of red pine cones (*Pinus resinosa* Ait.). Can. J. Bot. 34: 37-36.

Lyr, V.E.; Hoffman, G. 1964. Uber den Einfluss der Mineralsalzernährung auf die Frubfruktifikation von *Cryptomeria japonica* (L.F.D.) Don. Flora 154: 189-208.

MacDonald, A.D.; Mothersill, D.H. 1983. Shoot development in *Betula papyrifera*. I. Short-shoot organogenesis. Can. J. Bot. 61: 3049-3065.

MacDonald, A.D.; Mothersill, D.H.; Caesar, J.C. 1984. Shoot development in *Betula papyrifera*. III. Long-shoot organogenesis. Can. J. Bot. 62: 437-445.

MacDonald, J.E. 1962. Collection and washout of airborne pollens and spores by raindrops. Science 135: 435-437.

McDonald, P.M. 1969. Silvical characteristics of California black oak (*Quercus kelloggii* Newb.). USDA Forest Serv. Res. Paper No. PSW-53, 20 p.

McElwee, R.L. 1970. Radioactive tracer techniques for pine pollen flight studies and an analysis of short-range pollen behaviour. Ph.D. Thesis, N.C. State Univ., Raleigh. 97 p.

McKee, H.S. 1958. Protein metabolism in flowers. Plant Physiol. 8: 554-563.

McLemore, B.F. 1975. Cone and seed characteristics of fertilized and unfertilized longleaf pines. USDA Forest Serv. Res. Pap. No. SO-109. 10 p.

McMullan, E.E. 1980. Effect of applied growth regulators on cone production in Douglas-fir, and relation of endogenous growth regulators to cone production capacity. Can. J. Forest Res. 10: 405-414.

McWilliam, J.R. 1958. The role of the micropyle in the pollination of *Pinus*. Bot. Gaz. 120: 109-117.

McWilliam, J.R. 1959a. Interspecific incompatibility in *Pinus*. Am. J. Bot. 46: 425-433.

McWilliam, J.R. 1959b. Bioelectrical phenomena in relation to pollination in *Pinus*. Silvae Genet. 8: 59-61.

McWilliam, J.R. 1960. Pollen germination of *Pinus* as affected by the environment. Forest Sci. 6: 27-39.

Maguire, W.P. 1956. Are ponderosa pine crops predictable? J. For. 54: 778-779.

Maheshwari, P. 1950. An introduction to the embryology of the Angiosperms. McGraw-Hill, New York. 453 p.

Maheshwari, P. ed. 1963. Recent advances in the embryology of Angiosperms. Internat. Soc. Plant Morphologists, Univ. of Delhi. 467 p.

Maheshwari, P.; Roy, S.K. 1951. The embryo sac of Salix. Phytomorphology 1: 70-72.

Makhmet, B.M.; Shlonchak, A.V. 1977. [Storage of pollen of some woody species by the deep freezing method.] Ukrainskii Botanichnyi Zhurnal 34: 195-197.

Malkonen, E. 1971. Fertilizer treatment and seed crop of Picea abies. Commun. Inst. For. Fenn. 73.4. 16 p.

Mameli, C.E. 1952. [The reserve substances of pollen and their phylogenetic, ecological and embryolical significance.] Nuovo Gior. Bot. Ital. 59: 1-26.

Mann, W.F., Jr.; Russell, T.E. 1957. Longleaf cone production doubled by ringing. Tree Planter's Notes 28: 6-7.

Marquard, R.D.; Hanover, J.W. 1984. Sexual zonation in the crown of Picea glauca and flowering response to exogenous $GA_{4/7}$. Can. J. Forest Res. 14: 27-30.

Martens, P.; Waterkeyn, L. 1962. Structure du pollen "aile" chez les conifères. Cellule 62: 172-222.

Mascarhenas, J.P.; Bell, E. 1969. Protein synthesis during germination of pollen: studies on polysome formation. Biochem. biophys. Acta. 179: 199-203.

Matthews, F.R.; Bramlett, D.L. 1981. Cyclone pollinator improves loblolly pine seed yields in controlled pollinations. Southern J. App. For. 5: 42-46.

Matthews, F.R.; Karus, J.F. 1981. Pollen storage. Pages 37-39 in Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv. Agric. Handbook No. 587.

Matthews, J.D. 1955. The influence of weather on the frequency of beech mast years in England. Forestry 28: 107-116.

Matthews, J.D. 1963. Factors affecting the production of seed by forest trees. For. Abs. 24(1): i-xiii.

Matyas, V. 1969. Weather influence on beech flowering. Pages 1407-1418 in Proc. 2nd FAO/IUFRO World Consult. For. Tree Breed. Washington.

Maurinj, A.M.; Kaurov, I.A. 1956. [Comparison of methods of determining the viability of tree pollen]. Bot. Z. 41: 81-84.

Meehan, Th. 1888. Contrib. life hist. plants. 3. Dichogamy and its significance. Proc. Acad. Nat. Sci. Phil. p. 391. Cited in Klaehn, F.U. 1961. Flower morphology and flower ecology with respect to forest tree breeding procedures. Recent Advances in Botany 2: 1666-1670.

Mehra, P.N.; Dogra, P.D. 1965. Normal and abnormal pollen grains in Abies pindrow (Royle) Spach. Palynological Bull. 1: 16-23.

Mehra, P.N.; Dogra, P.D. 1975. Embryogeny of Pinaceae. I. Proembryogeny. Proc. Indian Nat. Sci. Acad. 11, B.: 486-497.

Melchior, G.H. 1960. Girdling experiments to increase flowering ability in Japanese larch L. leptolepis (Sieb. et Zucc.) Gord. and European larch, L. decidua Mill. Silvae Genet. 9: 105-111.

Melchior, G.H. 1961a. The induction of flowering in Japanese larch graftings. Silvae Genet. 10: 20-27.

Melchior, G.H. 1961b. Experiments with girdling of grafts of European and Japanese larch (Larix decidua and Larix leptolepis). Silvae Genet. 10: 97-124.

Melchior, G.H. 1968. [Experiments on flower stimulation in Douglas-fir grafts.] Silvae Genet. 17: 26-31.

Melchior, G.H.; Heitmuller, H.H. 1961. Increasing the number of male flowers in grafts of *Pinus sylvestris* by pruning. Silvae Genet. 10: 180-186.

Mergen, F. 1962. Flower induction in young pine trees. *in* Proc. 13th IUFRO Congress, Vienna, 1961. Part 2,1. 5 p.

Mergen, F.; Johansen, T.S. 1963. Effect of ionizing radiation on microsporogenesis in *Pinus rigida* (Mills). Rad. Bot. 3: 321-331.

Mergen, F.; Lester, D.T. 1961. Microsporogenesis in *Abies*. Silvae Genet. 10: 146-156.

Mergen, R.; Rossoll, H.; Pomeroy, K. 1955. How to control the pollination of slash and longleaf pine. USDA Forest Serv. Res. Paper No. SE-58. 14 p.

Mergen, F.; Stairs, G.R.; Snyder, E.B. 1963. Microsporogenesis in *Pinus echinata* and *Pinus taeda*. Silvae Genet. 12: 127-129.

Mergen, F.; Burley, J.; Furnival, G.M. 1965. Embryo and seedling development in *Picea glauca* (Moench) Voss after self-, cross-, and wind pollination. Silvae Genet. 14: 188-194.

Merkle, S.A.; Feret, P.P.; Croxdale, J.G.; Sharik, T.L. 1980. Development of floral primordia in white oak. Forest Sci. 26: 238-250.

Meyen, S.V. 1984. Basic features of Gymnosperm systematics and phylogeny as evidenced by the fossil record. Bot. Rev. 50: 1-111.

Michalski, L. 1967. Growth regulators in the pollen of pine. Acta Soc. Bot. Pol. 36: 475-481.

Migita, K. 1960a. [Effects of soil water moisture on the flower bud formation of sugi seedlings.] J. Jpn. For. Soc. 42: 27-28.

Migita, K. 1960b. Influence of shading and daylength on the flower bud formation of Sugi (*Cryptomeria japonica*) seedlings. J. Jpn. For. Soc. 42: 49-51.

Mikami, S.; Asakawa, S,; Iizuka, M.; Yokoyama, T.; Nagao, A.; Takehana, S.; Kaneko, T. 1979. Flower induction in Japanese larch, *Larix leptolepis* Gord. Jpn. For. Prod. Res. Inst. For. Bull. 307: 9-24.

Mikkola, L. 1969. Observations on interspecific sterility in *Picea*. Ann. Bot. Fenn. 6: 285-339.

Miller, C.N., Jr. 1977. Mesozoic conifers. Bot. Rev. 43: 217-280.

Miller, C.N., Jr. 1982. Current status of Paleozoic and Mesozoic conifers. Paleobot. Palynol. 37: 99-114.

Mirov, N.T. 1951. Inducing early production of pine pollen. USDA Forest Serv., Calif. For. & Range Exp. Stn. For. Res. Notes No. 80. 3 p.

Mirov, N.T. 1956. Photoperiod and flowering of pines. Forest Sci. 2: 328-332.

Mogensen, H.L. 1975. Ovule abortion in *Quercus* (Fagaceae). Am. J. Bot. 62: 160-165.

Moir, R.B.; Fox, D.P. 1975. Male meiosis in Sitka spruce, *Picea sitchensis* (Bong.) Carr. Silvae Genet. 24: 187-192.

Mosely, M.F. 1943. Contributions to the life history, morphology, and physiology of *Widdringtonia cupressoides*. Lloydia 6: 109-132.

Muelder, D.W.; Hansen, J.H. 1961. Observations on cone bearing of *Sequoia sempirvirens*. Calif. Agric. Exp. Stn. Berkeley, Calif. Bull. No. 26. 6 p.

Mulcahy, G.B.; Mulcahy, D.L.; Pfahler, P.L. 1982. The effect of delayed pollination in *Petunia hybrida*. Acta. Bot. Neerl. 31: 97-103.

Muller, G. 1977. Cross-fertilization in a conifer stand inferred from enzyme gene-markers in seeds. Silvae Genet. 126: 223-226.

Muller-Olsen, C.; Simak, M. 1954. X-ray photography employed in germination analysis of Scots pine (Pinus sylvestris L.). Medd. Stat. Skogsforskn-Inst. 19 p.

Nagao, A. 1983a. [Effects of different light spectral compositions on floral initiation in Chamaecyparis obtusa seedlings under various temperature conditions.] J. Jpn. For. Soc. 65: 233-236.

Nagao, A. 1983b. [Differences of flower initiation of Cryptomeria japonica under various alternating temperatures.] J. Jpn. For. Soc. 65: 335-338.

Nagao, A.; Sasaki, S. 1981. Effects of temperature and light on promotion of strobilus of Cryptomeria japonica and Chamaecyparis obtusa. Pages 103-114 in Krugman, S.L.; Katsuta, M., eds. Proc. Symp. Flowering Physiology. 17th IUFRO World Cong., Kyoto, Japan. Jpn. For. Tree Breeding Assoc., Tokyo.

Nagaraj, M. 1952. Floral morphology of Populus deltoides and P. tremuloides. Bot. Gaz. 114: 222-243.

Nakai, I.; Fujimoto, H.; Inamori, Y. 1976. [Studies on the cross-breeding of genus Pinus. (II). On the process of fertilization in the interspecific pollination of Pinus thunbergii Parl. with P. densiflora Sieb. et Zucc. and other pine species.] Kyoto Univ. Jpn. For. Bull. 48: 31-45.

Nanda, K.K. 1962. Some observations on growth, branching behaviour and flowering of Teak (Tectonia grandis L.F.) in relation to light. Indian Forester 88: 207-218.

Nanson, A. 1965. Stimulation de la production de strobiles femelles dans un verger à graines de Pinus sylvestris L. par application d'engras. Silvae Genet. 14: 94-97.

Nemec, A. 1956. [Improving the seeding of Fagus sylvatica by soil improvement.] Práce výzkum Ust. lesn. CSR 11: 5-25.

Nettancourt, D. de. 1977. Incompatibility in Angiosperms. Springer Verlag, New York.

Nielsen, P.C.; Schaffalitzky de Muckadell, M. 1954. Flower observations and controlled pollinations in Fagus. Silvae Genet. 3: 6-17.

Nienstaedt, H.; Kriebel, H.B. 1955. Controlled pollination of eastern hemlock. Forest Sci. 1: 115-120.

Niklas, K.J. 1981. Airflow patterns around some early seed plant ovules and cupules: Implications concerning efficiency in wind pollination. Am. J. Bot. 68: 635-650.

Niklas, K.J. 1982. Simulated and empiric wind pollination patterns of conifer ovulate cones. Proc. Natl. Acad. Sci. USA 79: 510-514.

Niklas, K.J. 1983. The influence of Paleozoic ovule and cupule morphologies on wind pollination. Evolution 37: 968-986.

Niklas, K.J. 1984. The motion of wind-borne pollen grains around conifer ovulate cones: Implications for wind pollination. Am. J. Bot. 71: 356-374.

Niklas, K.J.; Norstog, K. 1984. Aerodynamics and pollen grain depositional patterns on cycad megastrobili: Implications for the reproduction of three Cycad genera (Cycas, Dioon, and Zamia). Bot. Gaz. 145: 92-104.

Niklas, K.J.; Paw U, K.T. 1982. Pollination and airflow patterns around ovulate cones. Science 217: 442-444.

Niklas, K.J.; Paw U, K.T. 1983. Conifer ouvlate cone morphology: Implications on pollen impaction patterns. Am. J. Bot. 70: 568-577.

Nitsch, J.P. 1963. Fruit development. Pages 361-394 in Maheshwari, P., ed. Recent advances in the embryology of Angiosperms. Catholic Press, Ranchi, India.

Nygaard, P. 1973. Nucleotide metabolism during pine pollen germination. Physiol. Plant. 28: 361-371.

Ogden, E.C.; Lewis, D.M. 1960. Airborne pollen and fungus spores of New York State. N.Y. State Mus., Bull. No. 378. 103 p.

Ohba, K.; Iwakawa, M.; Okada, Y.; Murai, M. 1971. Paternal transmission of a plastid anomaly in some reciprocal crosses of Sugi, *Cryptomeria japonica* D. Don. Silvae Genet. 20: 101-107.

Olsson, U. 1975. On the size and microstructure of pollen grains of *Quercus robur* and *Q. petraea* (Fagaceae). Bot. Notiser 128: 256-264.

Orr-Ewing, A.L. 1954. Inbreeding experiments with Douglas-fir. For. Chron. 30: 7-16.

Orr-Ewing, A.L. 1956. Controlled pollination techniques for the Douglas-fir. Forest Sci. 7: 251-257.

Orr-Ewing, A.L. 1957. A cytological study of the effects of self-pollination on *Pseudotsuga menziesii* (Mirb.) Franco. Silvae Genet. 6: 179-185.

Orr-Ewing, A.L. 1965. Inbreeding and single crossing in Douglas-fir. Forest Sci. 11: 279-290.

Ottley, A.M. 1909. The development of the gametophytes and fertilization in *Juniperus communis* and *Juniperus virginiana*. Bot. Gaz. 48: 31-46.

Owens, J.N. 1969. The relative importance of initiation and early development on cone production in Douglas-fir. Can. J. Bot. 47: 1039-1049.

Owens, J.N. 1973. Reproductive patterns in conifers. *In* Sexual reproduction of conifers. Academy of Sciences of the USSR. (Siberian branch, Biological Institute.) Forest and Wood Institute, All-Union symposium.

Owens, J.N. 1980. Reproductive biology of conifers. Pages 77-92 *in* Dancik, B.P.; Higgenbotham, K.O., eds. Proc. Sixth North Amer. Forest Biol. Workshop. Univ. Alta., Edmonton.

Owens, J.N. 1982. Pollen management through phenological control of development. Pages 39-48 *in* Pollard, D.F.W.; Edwards, D.G.; Yeatman, C.W., eds. Proc. 18th Mtg. Can. Tree Improv. Assoc. Pt. 2. Duncan B.C. 1981. Environ. Can. Can. For. Serv., Petawawa Nat. For. Inst.

Owens, J.N. 1984a. Bud development in mountain hemlock (*Tsuga mertensiana*). II. Cone-bud differentiation and predormancy development. Can. J. Bot. 62: 484-494.

Owens, J.N. 1984b. Bud development in grand fir (*Abies grandis*). Can. J. Forest Res. 14: 575-588.

Owens, J.N.; Blake, M.D. 1983. Pollen morphology and development of the pollination mechanisms in *Tsuga heterophylla* and *T. mertensiana*. Can. J. Bot. 61: 3041-3048.

Owens, J.N.; Blake, M.D. 1984. The pollination mechanism of Sitka spruce (*Picea sitchensis*). Can. J. Bot. 62: 1136-1148.

Owens, J.N.; Molder, M. 1971a. Pollen development in Douglas-fir (*Pseudotsuga menziesii*). Can. J. Bot. 49: 1263-1266.

Owens, J.N.; Molder, M. 1971b. Meiosis in conifers: prolonged pachytene and diffuse diplotene stages. Can. J. Bot. 49: 2061-2064.

Owens, J.N.; Molder, M. 1974a. Bud development in western hemlock. II. Initiation and early development of pollen cones and seed cones. Can. J. Bot. 52: 283-294.

Owens, J.N.; Molder, M. 1974b. Yellow cedar cone production. B.C. Forest Serv. Res. Notes E.P. 730 No. 68. 4 pp.

Owens, J.N.; Molder, M. 1975a. Development of long-shoot terminal buds of *Pinus contorta* spp. *contorta*. Pages 85-104 *in* Daumgartner, D.M., ed. Proc. Management of Lodgepole Pine Ecosystems Symp. Washington State Univ.

Owens, J.N.; Molder, M. 1975b. Pollination, female gametophyte, and embryo and seed development in yellow cedar (*Chamaecyparis nootkatensis*). Can. J. Bot. 53: 186-199.

Owens, J.N.; Molder, M. 1975c. Sexual reproduction of mountain hemlock (*Tsuga mertensiana*). Can. J. Bot. 43: 1811-1826.

Owens, J.N.; Molder, M. 1976. Bud development in Sitka spruce. II. Cone differentiation and early development. Can. J. Bot. 54: 766-779.

Owens, J.N.; Molder, M. 1977a. Vegetative bud development and cone differentiation in *Abies amabilis*. Can. J. Bot. 55: 992-1008.

Owens, J.N.; Molder, M. 1977b. Development of long-shoot terminal buds of western white pine (*Pinus monticola*). Can. J. Bot. 55: 1308-1321.

Owens, J.N.; Molder, M. 1977c. Seed-cone differentiation and sexual reproduction in western white pine (*Pinus monticola*). Can. J. Bot. 55: 2574-2590.

Owens, J.N.; Molder, M. 1977d. Sexual reproduction of *Abies amabilis*. Can. J. Bot. 55: 2653-2667.

Owens, J.N.; Molder, M. 1977e. Bud development in *Picea glauca*. II. Cone differentiation and early development. Can. J. Bot. 55: 2746-2760.

Owens, J.N.; Molder, M. 1977f. Cone induction in yellow cypress (*Chamaecyparis nootkatensis*) by gibberellin A_3 and the subsequent development of seeds within the induced cones. Can. J. Forest Res. 7: 605-613.

Owens, J.N.; Molder, M. 1977g. The times and patterns of cone differentiation in western conifers. *In* Proc. Third World Consultation on Forest Tree Breeding. CSIRO, Canberra, Australia. 6 p.

Owens, J.N.; Molder, M. 1979a. Sexual reproduction of white spruce (*Picea glauca*). Can. J. Bot. 57: 152-169.

Owens, J.N.; Molder, M. 1979b. Bud development in *Larix occidentalis*. II. Cone differentiation and early development. Can. J. Bot. 57: 1557-1572.

Owens, J.N.; Molder, M. 1979c. Sexual reproduction of *Larix occidentalis*. Can. J. Bot. 57: 2673-2690.

Owens, J.N.; Molder, M. 1979d. The times and patterns of cone differentiation in western North American conifers. Pages 25-32 *in* Bonner, F., ed. Proc. Symp. Flowering and Seed Development in Trees. IUFRO. 1978. Miss. State Univ. USDA Forest Serv. Southern Forest Exper. Stn.

Owens, J.N.; Molder, M. 1980a. Sexual reproduction of Sitka spruce (*Picea sitchensis*). Can. J. Bot. 58: 886-901.

Owens, J.N.; Molder, M. 1980b. Sexual reproduction in western red cedar (*Thuja plicata*). Can. J. Bot. 58: 1376-1393.

Owens, J.N.; Molder, M. 1984a. The reproductive cycles of western red cedar and yellow cedar. B.C. Min. Forest Inf. Serv. Br. Victoria. 28 p.

Owens, J.N.; Molder, M. 1984b. The reproductive cycle of lodgepole pine. B.C. Min. Forest Inf. Serv. Br., Victoria. 29 p.

Owens, J.N.; Molder. 1984c. The reproductive cycle of interior spruce. B.C. Min. Forest Inf. Serv. Br., Victoria. 30 p.

Owens, J.N.; Molder, M. 1984d. The reproductive cycles of western and mountain hemlock. B.C. Min. Forest Inf. Serv. Br., Victoria. 34 p.

Owens, J.N.; Molder, M. 1985. The reproductive cycles of the true firs. B.C. Min. Forest Inf. Serv. Br., Victoria. (in press).

Owens, J.N.; Pharis, R.P. 1967. Initiation and ontogeny of the microsporangiata cone in *Cupressus arizonica* in response to gibberellin. Am. J. Bot. 54: 1260-1272.

Owens, J.N.; Pharis, R.P. 1971. Initiation and development of western red cedar cones in response to gibberellin induction and under natural conditions. Can. J. Bot. 49: 1165-1175.

Owens, J.N.; Simpson, S.J. 1982. Further observations on the pollination mechanism and seed production of Douglas-fir. Can. J. Forest Res. 12: 431-434.

Owens, J.N.; Singh, H. 1982. Vegetative bud development and the time and method of cone initiation in subalpine fir (*Abies lasiocarpa*). Can. J. Bot. 60. 2249-2262.

Owens, J.N.; Smith, F.H. 1964. The initiation and early development of the seed cone of Douglas-fir. Can. J. Bot. 42: 1031-1047.

Owens, J.N.; Smith, F.H. 1965. Development of the seed cone of Douglas-fir following dormancy. Can. J. Bot. 43: 317-332.

Owens, J.N.; Molder, M.; Langer, H. 1977. Bud development in *Picea glauca*. I. Annual growth cycle of vegetative buds and shoot elongation as they relate to date and temperature sums. Can. J. Bot. 55: 2728-2745.

Owens, J.N.; Simpson, S.; Molder, M. 1980. The pollination mechanism in yellow cypress (*Chamaecyparis nootkatensis*). Can. J. Forest Res. 10: 564-572.

Owens, J.N.; Simpson, S.J.; Molder, M. 1981a. The pollination mecnanism and the optimal time of pollination in Douglas-fir (*Pseudotsuga menziesii*). Can. J. Forest Res. 11: 36-50.

Owens, J.N.; Simpson, S.J.; Molder, M. 1981b. Sexual reproduction of *Pinus contorta*. I. Pollen development, the pollination mechanism, and early ovule development. Can. J. Bot. 59: 1828-1843.

Owens, J.N.; Simpson, S.J.; Molder, M. 1982. Sexual reproduction of *Pinus contorta*. II. Postdormancy ovule, embryo, and seed development. Can. J. Bot. 60: 2071-2083.

Owens, J.N.; Webber, J.E.; Ross, S.D.; Pharis, R.P. 1985. Interaction between gibberellin $A_{4/7}$ and rootpruning on the reproductive and vegetative processes in Douglas-fir. III. Effects on anatomy of shoot elongation and terminal bud development. Can. J. Forest Res. 15: 354-364.

Owens, J.N.; Webber, J.E.; Ross, S.D.; Pharis, R.P. Interaction between gibberellin $A_{4/7}$ and root-pruning on the reproductive and vegetative procession Dougls-fir. IV. Effects on anatomy of lateral bud development. Can. J. Forest Res. (Submitted).

Owston, P.W. 1969. The shoot apex in eastern white pine: its structure, seasonal development and variation within the crown. Can. J. Bot. 47: 1181-1188.

Perfil'ev, V.W. 1962. Relation between calcium content in the ash of apple shoots and the capacity of trees for forming fruit buds. Sov. Pl. Physiol. 9: 292-293.

Pettitt, J.M. 1977a. Detection in primitive gymnosperms of proteins and glycoproteins of possible significance in reproduction. Nature 266: 530-632.

Pettitt, J.M. 1977b. The megaspore wall in gymnosperms: ultrastructure in some zooidogamous forms. Proc. Roy. Soc. Lond. B. 195: 497-515.

Pettitt, J.M. 1979. Precipitation reactions occur between components of the ovule tissues in primitive gymnosperms. Ann. Bot. 44: 369-371.

Pettitt, J.M. 1982. Ultrastructural and immunocytochemical demonstration of gametophytic proteins in the pollen tube wall of the primitive gymnosperms *Cycas*. J. Cell. Sci. 57: 189-213.

Phares, R.E.; Rogers, N.F. 1962. Improving shortleaf pine seed production. J. For. 60: 322-324.

Pharis, R.P. 1976. Manipulation of flowering in conifers through the use of plant hormones. Pages 265-282 *in* Miksche, M.P., ed. Modern methods *in* forest genetics. Springer Verlag, New York.

Pharis, R.P. 1977. Interaction of native or exogenous plant hormones in the flowering of woody plants. Pages 343-360 *in* Schuttle, H.R.; Gross, D., eds. Proc. Conf. Regulation of developmental processes in plants. Halle, DDR.

Pharis, R.P.; Kuo, C.G. 1977. Physiology of gibberellins in conifers. Can. J. Forest Res. 7: 299-325.

Pharis, R.P.; Morf, W. 1967. Experiments on the precocious flowering of western red cedar and four species of *Cupressus* with gibberellins A_3 and $A_{4/7}$ mixture. Can. J. Bot. 45 1519-1524.

Pharis, R.P.; Morf, W. 1969. Precocious flowering of coastal and giant redwoods with gibberellin A_3, $A_{4/7}$, and A_{13}. BioScience 19: 719-720.

Pharis, R.P.; Morf, W. 1970. Sexuality in conifers: Effects of photoperiod and gibberellin concentration on the sex of gibberellin-induced strobili of western red cedar (*Thuja plicata* Donn.). Pages 85-89 *in* Zeszyty Naukowe Uniwersytetu Mikolaja Kopernika W. Toruniu, Nauki Matematyczmo-Przyrodnicze-Zeszyt 23 - Biologia.

Pharis, R.P.; Ross, S.D. 1976. Gibberellins: their potential uses in forestry. Outlook on Agriculture 9: 82-87.

Pharis, R.P.; Ross, S.D. 1985. The hormonal promotion of flowering in the Pinaceae. *In* Halevy, A.H., ed. Handbook of flowering. CRC Press Inc. Boca Raton, Florida. Vol. 5. (In press.)

Pharis, R.P.; Ruddat, M.D.E.; Phillips, C.C.; Heftmann, E. 1965. Precocious flowering of Arizona cypress with gibberellin. Can. J. Bot. 43: 923-927.

Pharis, R.P.; Crozier, A.; Reid, D.M.; Kuo, C.C. 1969. Activity of gibberellin A_{24} in 10 plant bioassays. Can. J. Bot. 47: 815-820.

Pharis, R.P.; Ruddat, M.D.E.; Glenn, J.L.; Morf, W. 1970. A quantitative requirement for long day in the induction of staminate strobili by gibberellin in the conifer *Cupressus arizonica*. Can. J. Bot. 48: 653-658.

Pharis, R.P., Wample, R.L.; Kamienska, A. 1975. Growth, development, and sexual differentiation in *Pinus* with emphasis on the role of the plant hormone, gibberellin. Pages 106-134 *in* Baumgartner, D.M., ed. Management of lodgepole pine ecosystems. Washington State Univ.

Pharis, R.P.; Ross, S.D.; McMullan, E. 1980. Promotion of flowering in the Pinaceae by gibberelins. III. Seedlings of Douglas-fir. Pnysiol. Plant. 50: 119-126.

Philippis, A. de; Magini, E.: Piussi, P. 1966. Biological effects of tapping research on *Pinus pinea*, *P. pinaster*, and *P. sylvestris*. Ann. Accad. Ital. Sci. For. 15: 435-570.

Philipson, J.J. 1983. The role of gibberellin $A_{4/7}$, heat and drought in the induction of flowering in Stika spruce. J. Exp. Bot. 34: 291-302.

Picton, J.M.; Steer, M.W. 1982. A model for the mechanism of tip extension in pollen tubes. J. Theor. Biol. 98: 15-20.

Pierard, D.; Jacqmard, A.; Bernier, G. 1977. Changes in the protein composition of the shoot apical bud of *Sinapsis alba* in transition to flowering. Physiol. Plant. 21: 254-258.

Pierard, D.; Jacqmard, A.; Bernier, G.; Salmon, J. 1980. Appearance and disappearance of proteins in the shoot apical meristem of *Sinapsis alba* in transition to flowering. Planta 150: 397-405.

Pitcher, J.A. 1972. Repeated fertilization fails to stimulate cone production in young loblolly pine stand. Tree Planter's Notes 23: 28-30.

Pjatnitsky, S.S. 1947. [On pollination in oaks and the germination of pollen on the stigmas]. Dokl. Akad. Nauk. S.S.S.R. 56: 545-547.

Plym Forshell, C. 1974. Seed development after self-pollination and cross-pollination of Scots pine, *Pinus sylvestris* L. Stud. For. Suec. 118: 1-37.

Pollard, D.F.W.; Portlock, F.T. 1981a. Effect of temperature on strobilus production in gibberellin-treated seedlings of western hemlock. Can. Forestry Serv. Res. Notes 1: 21-22.

Pollard, D.F.W.; Portlock, F.T. 1981b. Response of strobilus production to gibberellin and fertilizer treatment in a young western hemlock clone bank on western Vancouver Island. Can. Forestry Serv. Res. Notes. 1: 27-28.

Pollard, D.F.W.; Portlock, F.T. 1983. Timing and duration effects of gibberellin and fertilizer treatment on strobilus production in young western hemlock. Can. Forestry Serv. Res. Notes. 3: 3-5.

Pollard, D.F.W.; Portlock, F.T. 1984. The effects of photoperiod and temperature on gibberellin $A_{4/7}$ induced strobilus production of western hemlock. Can. J. Forest Res. 14: 291-294.

Pond, J.D. 1936. Girdling for seed production. J. For. 34: 78-79.

Ponder, F., Jr. 1979. Fertilization and release increase nut production of pole size black walnut. Pages 138-144 in Bonner, F., ed. Proc. Symp. Flowering and seed development in trees. IUFRO, 1978, Miss. State Univ. USDA Forest Serv., Southern For. Exper. Stn.

Popnikola, N. 1971. Study of the morphological and physiological characteristics of pollen of Abies alba in relation to its hybridization. Sum. List 95: 291-308.

Powell, G.R. 1970. Postdormancy development and growth of microsporangiate and magasporangiate strobili in Abies balsamea. Can. J. Bot. 48: 419-428.

Powell, G.R. 1972. Some observations on the transition from female bearing to male bearing in balsam fir. Pages 18-26 in Proc. 19th Northeast Forest Tree Improv. Conf., Orono, Maine.

Powell, G.R. 1974. Initiation and development of lateral buds in Abies balsamea. Can. J. Forest Res. 4: 458-469.

Powell, G.R. 1977a. Patterns of development in Abies balsamea crowns and effects of magastrobilus production on shoots and buds. Can. J. Forest Res. 7: 498-509.

Powell, G.R. 1977b. Bienniel strobilus production in balsam fir: a review of its morphogenesis and a discussion of its apparent physiological basis. Can. J. Forest Res. 7: 547-555.

Powell, G.R. 1979. Influence of position in the crown on cone size and seed yield of Abies balsamea. Pages 122-137 in Bonner, F., ed. Proc. Symp. Flowering and seed development in trees. IUFRO, 1978, Miss. State Univ. USDA Forest Serv. Southern For. Exper. Stn.

Powell, G.R.; Tosh, K.J.; Remphrey, W.R. 1984. Occurrence and distribution of cones borne laterally on long shoots of Larix laricina. Can. J. Bot. 62: 771-777.

Puritch, G.S. 1972 (rev. 1977). Cone production in conifers. A review of the literature and evaluation of research needs; with an economic analysis by Vyse, A.H. Dep. Environ. Can. Can. Forestry Serv., Pacific Forest Res. Centre, Rep. BC-X-65. 94 p.

Puritch, G.S.; McMullan, E.E.; Meagher, M.D.; Simmons, C.S. 1979. Hormonal enhancement of cone production in Douglas-fir grafts and seedlings. Can. J. Forest Res. 9: 193-200.

Quinn, C.J. 1966a. Gametophyte development and embryogeny in Podocarpaceae. III. Dacrydium bidwilli. Phytomorphology 16: 81-91.

Quinn, C.J. 1966b. Gametophyte development and embryogeny in the Podocarpaceae. IV. Dacrydium colensoi: General conclusions. Phytomorphology 16: 199-211.

Quirk, J.T. 1973. Cone production stimulus related to transplanting in red pine. Silvae Genet. 22: 71-72.

Rawal, S.K.; Agrawal, D.C. 1982. Gibberellins in flowering and non-flowering trees of Pinus caribaea. Indian Forester 108: 62-65.

Reeve, D.R.; Crozier, A. 1975. Gibberellin bioassays. Pages 35-64 in Krishnamoorthy, H.M., ed. Gibberellins and plant growth. John Wiley (Eastern) Ltd., New Delhi.

Regal, P.J. 1982. Pollination by winds and by animals: Ecology of geographic patterns. ARES 13: 497-524.

Rehfeldt, G.E.; Stage, A.R.; Bingham, R.T. 1971. Strobili development in western white pine: periodicity, prediction, and association with weather. Forest Sci. 17: 454-461.

Remrod, J. 1972. [Trials on the induction of flowering in a spruce seed orchard]. Pages 160-178 *in* Foreningen Skogstradsforadling Institutet for Skogsforbattring arsbok.

Reukema, D.L. 1961. Seed production of Douglas-fir increased by thinning. USDA Forest Serv., Pacific Northwest For. Range Exper. Stn. Res. Note No. 210, 5 p.

Ritchie, G.A. 1966. Phenology and ontogeny of the reproductive and primary vegetative structures of *Abies amabilis* and *Abies procera*. M.Sc. Thesis, Univ. Washington, Seattle. 115 p.

Ritchie, G.A.; Hinckley, T.M. 1975. The pressure chamber as an instrument for ecological research. Adv. Ecol. Res. 9: 165-254.

Romasov, N.W. 1957. [Laws of fruiting of the oaks.] Bot. Zh. SSSR 42: 41-56.

Romberger, J.A.; Gregory, R.A. 1974. Analytical morphogenesis and the physiology of flowering in trees. Pages 132-147 *in* Proc. 3rd. North American Forest Biol. Workshop.

Rosen, W.G. 1968. Ultrastructure and physiology of pollen. Ann. Rev. Plant Phys. 19: 435-462.

Ross, S.D. 1975. Effects of gibberellins and girdling on flowering and seed production by young Douglas-fir grafts. For. Res. Tech. Rep. 50/5330/75/14. Weyerhauser Corp. 8 p.

Ross, S.D. 1976. Differential flowering responses by young Douglas-fir and equisized seedlings to gibberellins and auxins. Acta Hort. 56: 163-168.

Ross, S.D. 1978. Influences of gibberellins and cultural practices on early flowering of Douglas-fir seedlings and grafts. Pages 997-1107 *in* Proc. Third World Consult. Forest Tree Breeding. 1977. Vol. 2, CSIRO, Canberra, Australia.

Ross, S.D. 1979. Evaluation of foliar spray formulations for seed orchard application of gibberellins. For. Res. Tech. Rep. 042-3001/79/10. Weyerhauser Corp. 12 p.

Ross, S.D. 1983a. Enhancement of shoot elongation in Douglas-fir by gibberellin $A_{4/7}$ and its relation to the hormonal promotion of flowering. Can. J. Forest Res. 13: 986-994.

Ross, S.D. 1983b. Optimization of gibberellin A_3 treatments for enhancing yields in seed orchards of western red cedar and yellow cedar. B.C. Min. Forests, Res. Br. Prog. Rep File No. EP 935.03. 26 p.

Ross, S.D.; Greenwood, M.S. 1979. Promotion of flowering in the Pinaceae by gibberellins. II. Grafts of mature and immature *Pinus taeda*. Physiol. Plant. 45: 207-210.

Ross, S.D.; Pharis, R.P. 1976a. Promotion of flowering in the Pinaceae by gibberellins. I. Sexually mature, nonflowering grafts of Douglas-fir. Physiol. Plant. 36: 182-186.

Ross, S.D.; Pharis, R.P. 1976b. Progress in the promotion of early flowering in Douglas-fir by gibberellins. For. Res. Tech. Rep. 50/3001/76/32. Weyerhauser Corp. 8 p.

Ross, S.D.; Pharis, R.P. 1982. Recent develoments in enhancement of seed production in conifers. Pages 26-38 *in* Pollard, D.F.W.; Edwards, D.G.; Yeatman, C.W., eds. Proc. 18th Mtg. Can. Tree Improvement Assoc., Duncan, B.C. 1981. Part 2. Can. For. Serv., Ottawa.

Ross, S.D.; Pharis, R.P. 1985. Flower induction in crop trees: Different mechanisms and techniques with special reference to conifers. *In* Cannell, M.G.R. et al., eds. Attributes of Trees as Crop Plants. Institute of Terrestrial Ecology, Monks Wood Exp. Sta., Abbots Ripton, Huntingdon, England. (In press).

Ross, S.D.; Pharis, R.P.; Heaman, J.C. 1980. Promotion of cone and seed production in grafted and seedling Douglas-fir seed orchards by application of gibberellin $A_{4/7}$ mixture. Can. J. Forest Res. 10: 464-469.

Ross, S.D.; Piesch, R.F.; Portlock, F.T. 1981. Promotion of cone and seed production in rooted ramets and seedlings of western hemlock by gibberellins and adjunct cultural treatments. Can. J. Forest Res. 11: 90-98.

Ross, S.D.; Pharis, R.P.; Binder, W.D. 1983. Growth regulators and conifers: Their physiology and potential uses in forestry. Pages 35-78 *in* Nickell, L.G., ed. Plant Growth Regulating Chemicals, Vol. 2. CRC Press, Boca Raton, Fla.

Ross, S.D.; Bollman, M.; Pharis, R.P.; Sweet, G.B. 1984. Gibberellin $A_{4/7}$ and the promotion of flowering in a conifer, *Pinus radiata*: Effects on the partitioning of photoassimilate within the bud during promordia initiation. Plant Physiol. (in press).

Ross, S.D.; Webber, J.E.; Pharis, R.P.; Owens. J.N. 1985. Interaction between gibberellin $A_{4/7}$ and root pruning on the reproductive and vegetative process in Douglas-fir. I. Effects on flowering. Can. J. Forest Res. 15: 341-347.

Rottink, B.A. 1982. Flower induction in rooted cuttings of western hemlock. Abstract No. 7 *in* Proc. Western For. Genet. Assoc. Ann. Mtg. 1982, Corvallis, Ore.

Roy Chowdhury, C. 1961. The morphology and embryology of *Cedrus deodara* Loud. Phytomorphology 11: 283-304.

Roy Chowdhury, C. 1962. The embryogeny of conifers: A review. Phytomorphology 12: 313-338.

Ryynanen, L. 1978. [Quality of pollen from Finnish broadleaves]. Folia Forestalia, Inst. For. Fenn. No.366. 12 p.

Sacher, J.A. 1954. Structure and seasonal activity of the shoot apices of *Pinus lambertiana* and *Pinus ponderosa*. Am. J. Bot. 41: 749-759.

Sachs, R.M. 1977. Nutrient diversion: an hypothesis to explain the chemical control of flowering. Hort. Sci. 12: 220-222.

Saito, H.; Takeoka, M. 1983. [Pollen production in 60-year old plantations of *Chamaecyparis obtusa*.] J. Jpn. Ecol. 33: 365-373.

Sarvas, R. 1957. Studies on the seed setting of Norway spruce. Medd. Nor. Skogsforøksvers 14: 529-556.

Sarvas, R. 1962. Investigations on the flowering and seed crop of *Pinus sylvestris*. Inst. For. Fenn. 53: 1-198.

Sarvas, R. 1965. The annual period of development of forest trees. Tiedeaketamia. Sitzungsber. Finn. Acad. Wiss. 1965: 211-231.

Sarvas, R. 1967. Pollen dispersal within and between subpopulations: role of isolation and migration in microevolution of tree species. Pages 332-345 *in* Proc. 14th Congr. IUFRO. Part 3.

Sarvas, R. 1968. Investigations on the flowering and seed crop of *Picea abies*. Commun. Inst. For. Fenn. 67. 84 p.

Sarvas, R. 1972. Investigations on the annual cycle of development of forest trees: Active period. Commun. Inst. For. Fenn. 76: 1-110.

Sarvas, R. 1974. Investigations on the annual cycle of development of forest trees. II. Autumn dormancy and winter dormancy. Commun. Inst. Forest. Fenn. 84: 1-101.

Sato, K. 1963. Some physiological actions of gibberellins on forest trees. FAO World Consult. For. Genet., Stockholm. FAO/FORGEN 63/-5/2:1-11.

Scheffer, T.C.; Hedgcock, G.G. 1955. Injury to northwestern forest trees by SO_2 from smelters. USDA Forest Serv. Tech. Bull. No. 1117. 19 p.

Schmidtling, R.C. 1971. Cultivating and fertilizing stimulate precocious flowering in loblolly pines. Silvae Genet. 20: 220-221.

Schmidtling, R.C. 1974. Fruitfulness in conifers: nitrogen, carbohydrate, and genetic control. Pages 148-164 *in* Proc. Third North Am. For. Biol. Wkshp. Colorado State Univ., Fort Collins.

Schmidtling, R.C. 1975. Fertilizer timing and formulation affect flowering in a loblolly pine seed orchard. Pages 153-160 *in* Proc. 13th Southern For. Tree Impr. Conf. 1975.

Schnarf, K. 1933. Embryologie der Gymnospermen. Gebrüder Brontraeger, Berlin.

Schopf, J.M. 1943. The embryology of *Larix*. Ill. Biol. Monogr. 19: 97 p.

Schopmeyer, C.S. 1974. Seeds of woody plants in the United States. USDA Forest Serv. Handbook No. 450. 883 p.

Schubert, G.H. 1956. Effect of fertilizer on cone production of sugar pine. USDA Forest Serv. Central Forest Res. Exper. Stn. For. Res. Note No. 116. 4 p.

Schultz, R.P. 1971. Stimulation of flower and seed production in a young slash pine orchard. USDA Forest Serv., Res. Paper No. SE 91. 10 p.

Schwabe, W.W.; Al-Doori, A.H. 1973. Analysis of a juvenile-like condition affecting flowering in the black current (*Ribes nigrum*). J. Exp. Bot. 82: 969-981.

Searle, N.E. 1965. Physiology of flowering. Ann. Rev. Pl. Physiol. 16: 97-118.

Seido, K. 1979. [Formation and development of pollen and method for pollen collection in *Abies veitchii* Lindl.]. Yamanaski Pref. For. Exper. Stn. Bull. 15: 10-20.

Seido, K.; Osada, T. 1979. [Morphological development of female flower buds in *Abies veitcheii* Lindl.]. Yamanaski Pref. For. Exper. Stn. Bull. 15: 1-9.

Seitz, F.W. 1958. Frühtreibversuche mit Blühreisern der Aspe. Silvae Genet. 7: 102-195.

Sharp, W.M.; Chisman, H.H. 1961. Flowering and fruiting in the white oaks. I. Staminate flowering through pollen dispersal. Ecology 42: 365-372.

Shaw, G.R. 1914. The genus *Pinus*. Publ. Arnold Arboretum, Cambridge, Mass. 96 p.

Shearer, R.C.; Schmidt, W.C. 1970. Natural regeneration in ponderosa pine forests of western Montana. USDA Forest Serv. Res. Paper No. INT-86. 19 p.

Shen, H.H.; Rudin, D.; Lindgren, D. 1981. Study of the pollination pattern in a Scots pine seed orchard by means of isozyme analysis. Silvae Genet. 30: 7-15.

Shoulders, E. 1967. Fertilizer application, inherent fruitfulness, and rainfall affect flowering of longleaf pine. Forest Sci. 13: 376-383.

Shoulders, E. 1968. Fertilization increases longleaf and slash pine flower and cone crops in Louisiana. J. For. 66: 193-197.

Sidhu, S.S. 1983. Effects of simulated acid rain on pollen germination and pollen tube growth of white spruce (*Picea glauca*). Can. J. Bot. 61: 3095-3099.

Silen, R.R. 1962. Pollen dispersal conditions for Douglas-fir. J. For. 60: 790-795.

Silen, R.R. 1963. Effect of altitude on factors of pollen contamination of Douglas-fir seed orchards. J. For. 61: 281-283.

Silen, R.R. 1973. July-stimulated flowering in Douglas-fir. Forest Sci. 19: 288-290.

Simak, M. 1966. Mognadsprocessen hos tallfrö (*Pinus sylvestris* L.). Sveriges Skogsvförb. Tidskr. 4: 411-426. (Dep. For. Gen., Swedish Roy. Coll. For., Res. Notes No. 2.)

Simak, M. 1979. Precocious flower induction in *Pinus sylvestris*. Pages 63-76 *in* Bonner, F., ed. Flowering and seed development in trees. IUFRO, 1978. Miss. State Univ. USDA Forest Service, Southern For. Exper. Stn.

Simak, M.; Gustafsson, A. 1953a. Rontgenfotogratering av skogsträdsfoö. Skogn 5: 1-4.

Simak, M.; Gustafsson, A. 1953b. X-ray photography and sensitivity in forest tree species. Kereditas 39: 458-468.

Simak, M.; Gustafsson, A. 1954. [Seed properties in mother trees and grafts of Scots pine]. Medd. Stat. Skogsforskn.-inst. 42: 2:1-73.

Simak, M.; Gustafsson, A. 1959. Röntgenanalys och det norrländska tallfröets kvalitesförbättring. Sv. Skogsv. fören. Tidskr. 57: 475-486.

Simak, M.; Kamra, S.K. 1963. Comparative studies on Scots pine seed germinability with tetrazolium and X-ray contrast method. Proc. Internat. Seed Testing Assn. 28: 3-18.

Simak, M.; Gustafsson, A.; Rautenberg, W. 1974. Meiosis and pollen formation in haploid Thuja plicata Oud. Hereditas 76: 227-238.

Simola, L.K. 1974. The ultrastructure of dry and germinating seeds of Pinus sylvestris L. Acta Bot. Finn. 103: 1-31.

Simpson, J.D.; Powell, G.R. 1981. Some factors influencing cone production on young black spruce in New Brunswick. For. Chron. 57: 267-269.

Singh, H. 1961. The life history and systematic position of Cephalotaxus drupacea Sieb. et Zucc. Phytomorphology 11: 153-197.

Singh, H. 1964. The proembryogeny of Cephalotaxus drupacea - A confirmation. Phytomorphology 14: 527-529.

Singh, H. 1978. Embryology of Gymnosperms. Gebrüder Borntraeger, Berlin. 302 p.

Singh, H.; Johri, B.M. 1972. Development of Gymnosperm seeds. Pages 21-75 in Kozlowski, T.T., ed. Seed Biology. Vol. 1 Academic Press, New York.

Singh, H.; Owens, J.N. 1981a. Sexual reproduction of Engelmann spruce (Picea engelmannii). Can. J. Bot. 59: 793-810.

Singh, H.; Owens, J.N. 1981b. Sexual reproduction in subalpine fir (Abies lasiocarpa). Can. J. Bot. 59: 2650-2666.

Singh, H.; Owens, J.N. 1982. Sexual reproduction in grand fir (Abies grandis). Can. J. Bot. 60: 2197-2214.

Singh, H.; Owens, J.N.; Dietrich, H.F. 1983. Ultrastructure and development of the microsporangium of Pseudotsuga menziesii (Mirb.) Franco. I. Dormant stages. Am. J. Bot. 70: 1272-1280.

Smith, J.H.G.; Walters, J.; Kozak, A. 1968. Influences of fertilizers on cone production and growth of young Douglas-fir, western hemlock and western red cedar on the U.B.C. research forest. Univ. British Columbia Fac. For. Bull. No. 5.

Smith, W.H.; Stanley, R.G. 1969. Cone distribution in crowns of slash pine (Pinus elliottii Englem.) in relation to stem, crown, and wood increment. Silvae Genet. 18: 86-91.

Snyder, E.B.; Clausen, K.E. 1974. Pollen handling. Pages 75-97 in Schopmeyer, C.S., ed. Seeds of woody plants in the United States. USDA Forest Serv. Agric. Handbook No. 450.

Somerville, J.G.; Sweet, G.B. 1978. Artificial pollination of Pinus radiata. Forest Res. Inst., Rotorua, For. Prod. Div., Genetics & Tree Improvement Int. Rep. No. 148. 10 p.

Sorensen, F.C. 1972. The seed orchard tree as a pollen sampler: A model and example. USDA Forest Serv. Res. Note No. PNW-175. 11 p.

Sorensen, F.C. 1982. The roles of polyembryony and embryo viability in the genetic system of conifers. Evolution 36: 725-733.

Sprague, J.R.; Johnson, V.W. 1977. Extraction and storage of loblolly pine (Pinus taeda) pollen. Pages 20-27 in Proc. 14th South. Forest Tree Improv. Conf.

Sprague, J.R.; Snyder, E.B. 1981. Extracting and drying pine pollen. Pages 33-36 in Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv. Agric. Handbook No. 587.

Sprague, J.; Jett, J.B.; Zobel, B. 1979. The management of southern pine seed orchards to increase seed production. Pages 145-162 in Bonner, F., ed. Proc. Symp. Flowering and Seed Development in Trees. IUFRO, 1978, Miss. State Univ. USDA Forest Serv., Southern For. Exper. Stn.

Squillance, A.E.; Long, E.M. 1981. Proportion of pollen from nonorchard sources. Pages 15-19 *in* Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv. Agri. Handbook No. 587.

Stairs, G.R. 1964. Microsporogenesis and embryogenesis in *Quercus*. Bot. Gaz. 125: 115-121.

Stanley, R.G. 1971. Pollen chemistry and tub growth. Pages 131-155 *in* Heslop-Harrison, J., ed. Pollen development and physiology. Butterworths, London.

Stanley, R.G.; Kirby, E.G. 1973. Shedding of pollen and seeds. Pages 295-340 *in* Kozlowski, T.T., ed. Shedding of plant parts. Academic Press, N.Y.

Stanley, R.G.; Linskens, H.F. 1974. Pollen: Biology, biochemistry, and management. Springer Verlag, New York. 307 p.

Stanley, R.G.; Poostchi, I. 1962. Endogenous carbohydrates, organic acids, and pine pollen viability. Silvae Genet. 11: 1-3.

Stanley, R.G.; Young, L.C.T. 1962. Desoxyribonucleic acid synthesis or turnover in non-dividing cells of *Pinus*. Nature 196: 1228-1229.

Starcenko, I.I. 1964. [Earlier fruiting of *Picea abies*]. Bot. Z. 49: 132-133.

Steffen, K. 1963. Fertilization. Pages 105-134 *in* Maheshwari, P., ed. Recent advances in the embryology of Angiosperms. Catholic Press, Ranchi, India.

Steinbrenner, E.C.; Duffield, J.W.; Campbell, R.K. 1960. Increased cone production of young Douglas-fir following nitrogen and phosphorous fertilization. J. For. 58: 105-110.

Stephens, G.R., Jr. 1961. Flower induction in woody plants. Ph.D. Thesis. Fac. Forestry, Yale Univ. 75 p.

Stephens, G.R., Jr. 1964. Stimulation of flowering in eastern white pine. Forest Sci. 10: 28-34.

Stephenson, A.G. 1980. Fruit set, herbivory, fruit reduction, and the fruiting strategy of *Catalpa speciosa* (Bignoniaceae). Ecology 61: 57-64.

Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Ann. Rev. Ecol. Syst. 12: 253-279.

Stephenson, A.G.; Bertin, R.I. 1983. Male competition, female choice, and sexual selection in plants. Pages 109-149 *in* Real, L., ed. Pollination Biology. Academic Press, Toronto.

Sterling, C. 1963. Structure of the male gametophyte in gymnosperms. Biol. Rev. 38: 167-203.

Stettler, R.F. 1968. Irradiated mentor pollen: its use in remote hybridization of black cottonwood. Nature 219: 746-747.

Stettler, R.F.; Bawa, S.K. 1971. Experimental induction of haploid parthenogenesis in black cottonwood (*Populus trichocarpa* T. & G. ex Hook.) Silvae Genet. 20: 15-25.

Stettler, R.F.; Koster, R.; Steenackers, V. 1980. Interspecific crossability studies in poplars. Theor. Appl. Genet. 58: 273-282.

Stoate, T.N.; Mahood, I.; Crossin, E.C. 1961. Cone production in Douglas-fir (*Pseudotsuga menziesii*). Emp. For. Rev. 40: 104-110.

Sucoff, E. 1971. Timing and rate of bud formation in *Pinus resinosa*. Can. J. Bot. 49: 1821-1832.

Sulkinoja, M.; Valanne, T. 1980. Polyembryony and abnormal germination in *Betula pubescens* subsp. *tortuosa*. Rep. Kevo Subarctic Res. Sta. Finland. 16: 31-37.

Summerville, K.O.; Jett, J.B.; Davey, C.B. 1979. Conelet abortion in a longleaf seed orchard: fertilization and irrigation effects. Pages 187-196 *in* Bonner, F., ed. Proc. Symp. Flowering and seed development in trees. IUFRO, 1978, Miss. State Univ. USDA Forest Service, Southern For. Exper. Stn.

Sweet, G.B. 1973. Shedding of reproductive structures in forest trees.

Pages 341-382 in Kozlowski, T.T., ed. Shedding of plant parts. Academic Press, New York.

Sweet, G.B. 1979. A physiological study of seed cone production in Pinus radiata. N.Z. J. For. Sci. 9: 20-33.

Sweet, G.B.; Bollman, M.P. 1970. Investigations into the causes of conelet drop in Pinus radiata in New Zealand. IUFRO, Sect. 22. Sexual Reprod. of For. Trees, Finland. N.Z. For. Ser. Rep. No. 439. ODC 161-166.

Sweet, G.B.; Bollman, M.P. 1971. Seasonal growth of the female strobilus in Pinus radiata. N.Z. J. For. Sci. 1: 15-21.

Sweet, G.B.; Bollman, M.D. 1972. Regional variation in Douglas-fir seed yields. N.Z. J. For. 17: 74-80.

Sweet, G.B.; Hong, S.O. 1975. Do gibberellins increase flower production in Pinus radiata? N.Z. For. Serv., Genet. & Tree Improvement Div. Int. Rep. No. 89. 10 p.

Sweet, G.B.; Hong, S.O. 1978. The role of nitrogen in relation to cone production in Pinus radiata. N.Z. J. For. Sci. 8: 225-238.

Sweet, G.B.; Krugman, S.L. 1978. Flowering and seed production problems - a new concept of seed orchards. Pages 749 759 in Proc. Third World Consult For. Tree Breeding. Vol. 2. 1977, CSIRO, Canberra, Australia.

Sweet, G.B.; Lewis, P.N. 1969. A diffusible auxin from Pinus radiata pollen and its possible role in stimulating ovule development. Planta 89: 380-384.

Sweet, G.B.; Lewis, P.N. 1971. Plant growth substances in pollen of Pinus radiata at different levels of germination. N.Z. J. Bot. 9: 146-156.

Sweet, G.B.; Will, G.M. 1965. Precocious male cone production associated with low nutrient status in clones of Pinus radiata. Nature 196: 739.

Sziklai, O. 1963. Variation and inheritance of some physiological and morphological traits in Pseudotsuga menziesii (Mirb.) Franco. Ph.D. Thesis. University of British Columbia, Vancouver.

Tahara, M. 1937. Contributions to the morphology of Sciadopitys verticillata. Cytologia: Fujii Jubilee Vol., 14-19.

Tahara, J. 1940. [Embryogeny of Torreya nucifera.] Bot. Mag., Tokyo 54: 469-472.

Takao, A. 1960. [Histochemical studies on embryogenesis of Pinus thunbergii.] Parl. Bot. Mag., Tokyo 73: 379-388.

Tanaka, K. 1956. The pollen germination and pollen tube growth in Pinus densiflora Sieb. et Zucc. II. The tube growth and tube nucleus. Tohoku Univ. Sci. Rep. Biol. 22: 219-224.

Tauber, H. 1965. Differential pollen dispersion and the interpretation of pollen diagrams. Dan. Geol. Unders. [Afh.], Rache 2 No. 89: 1-70.

Tauber, H. 1967. Differential pollen dispersion and filtration. Pages 131-141 in Cushing, E.J.; Wright, H.E., eds. Quaternary Paleoecology. Yale Univ. Press, New Haven, Conn.

Tauber, H. 1977. Investigations of aerial pollen transport in a forested area. Dan. Bot. Ark. 32: 1-121.

Thomas, M.J.; Chesnoy, L. 1969. Observations relatives aux mitochondries Feulgen positives de la zone périnucléaire de l'oosphere du Pseudotsuga menziesii (Mirb.) Franco. Rev. Cytol. Biol. Végét. 32: 165-182.

Thomson, R.B. 1945. "Polyembryony". Sexual and asexual embryo initiation and food supply. Trans. Roy. Soc. Can. Ser. 3. 39: 143-169.

Tompsett, P.B. 1977. Studies of growth and flowering in Picea sitchensis (Bong.) Carr. 1. Effects of growth regulator applications to mature scions on seedling rootstocks. Ann. Bot. 41: 1171-1178.

Tompsett, P.B. 1978a. Studies of growth and flowering in Picea sitchensis (Bong.) Carr. 2. Initiation and development of male, female, and vegetative buds. Ann. Bot. 42: 889-900.

Tompsett, P.B. 1978b. Effects of environmental and growth regulator treatments on the flowering of mature Sitka spruce. Pages 75-81 *in* Proc. Joint BCPC and BPGPG Symp. on Opportunities for Chemical Plant Growth Regulation. Oxford, England.

Tompsett, P.B.; Fletcher, A.M. 1977. Increased flowering of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) in a polythene house. Silvae Genet. 26: 84-86.

Tompsett, P.B.; Fletcher, A.M. 1979. Promotion of flowering on mature *Picea sitchensis* by gibberellin and environmental treatments. The influence of timing and hormonal concentration. Physiol. Plant. 45: 112-116.

Tsukada, M. 1982. [Pollen deposition of *Alnus rubra* Bong.] Jpn. J. Ecol. 32: 405-414.

Turkel, H.S.; Rebuck, A.L.; Grove, A.R., Jr. 1955. Floral morphology of white oak. Pacific Agric. Exper. Stn. Bull. 593: 15 p.

Van Den Berg, D.A.; Lanner, R.M. 1971. Bud development in lodgepole pine. Forest Sci. 17: 479-486.

Van Vredenburch, C.L.H.; La Bastide, J.G.A. 1969. The influence of meteorological factors on the cone crop of Douglas-fir in the Netherlands. Silvae Genet. 18: 182-186.

Van Wyk, G. 1981. Pollen management for Eucalypts. Pages 84-88 *in* Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv. Agric. Handbook No. 587.

Varnell, R.J. 1969. Female-strobilus production in a slash pine seed orchard following branch pruning. Pages 222-228 *in* Proc. Tenth Southern For. Tree Improv. Conf.

Varnell, R.J. 1970. Effects of branch girdling on the production of male and female strobili in longleaf pine. Forest Sci. 16: 195-196.

Varnell, R.J. 1976. Correlations between branch characteristics and production of female strobili in longleaf pine. Forest Sci. 22: 159-161.

Vasil, I.K. 1960. Studies on pollen germination of certain Cucurbitaceae. Am. J. Bot. 47: 239-247.

Vasil, I.K. 1978. The ultrastructure of pollen development in gymnosperms. Soc. Bot. Fr., Act. Bot. No. 1-2: 115-120.

Vasil, I.K.; Aldrich, H.C. 1970. A histochemical and ultrastructural study of the ontogeny and differentiation of pollen in *Podocarpus macrophyllus* D. Don. Protoplasma 71: 1-37.

Vasil, I.K.; Aldrich, H.C. 1971. Histochemistry and ultrastructure of pollen development in *Podocarpus macrophyllus* D. Don. Pages 70-74 *in* Heslop-Harrison, J., ed. Pollen development and physiology. Butterworth's, London.

Vasil, I.K.; Johri, M.M. 1964. The style, stigma, and pollen tube. Phytomorphology 14: 352-369.

Vidakovic, M.; Jurkovic-Bevilacqua, B. 1971. Development of ovules after interspecific hybridization of Austrian and Scots pines with gamma-irradiated pollen. Acta Biol. Jugoslav. Ser. Genetic 3: 119-130.

Villar, M.; Knox, R.B.; Dumas, C. 1984. Effective pollination period and nature of pollen-collecting aparatus in the Gymnosperm, *Larix leptolepis*. Ann. Bot. 53: 279-284.

Visser, T. 1955. Germination and storage of pollen. Mededel. Landbouwhogesch., Wageningen 55: 1-68.

Voronov, N.M. 1962. [Does resin tapping affect scots pine seed yield and quality?] Lesn. Hoz. 15: 80.

Wachter, H. 1959. [Observations on the fruiting of young larches in relation to damage by late frost.] Silvae Genet. 8: 105-106.

Wachter, H. 1962. [Further observations on the flowering and fruiting of young larch trees.] Silvae Genet. 11: 153-156.

Wakely, P.C.; Campbell, T.E. 1954. Some new pine pollination techniques. USDA Forest Serv. Southern For. Exper. Stn., Occas. Paper No. 36. 13 p.

Wallace, T. 1961. The diagnosis of mineral deficiencies in plants by visual symptoms. 2nd. ed. Chemical Publishing Co. Inc. N.Y. 125 p.

Wample, R.L.; Durley, R.C.; Pharis, R.P. 1975. Metabolism of gibberellin A_4 by vegetative shoots of Douglas-fir at three stages of ontogeny. Physiol. Plant. 35: 273-278.

Wang, B.S.P. 1975. Tree seed and pollen storage for genetic conservation: possibilities and limitations. Pages 93-103 *in* FAO/UNEP Report on a pilot study on the Methodology of Conservation of Forest Genetic Resources. FO: MISC/75/8.

Wang, C.W.; Perry, T.O.; Johnson, A.G. 1960. Pollen dispersion of slash pine (*Pinus elliottii* Engelm.) with special reference to seed orchard management. Silvae Genet. 9: 78-86.

Wardlaw, C.W. 1955. Embryogenesis in plants. Methuen. London. 381 p.

Wareing, P.F. 1958. Reproductive development in *Pinus sylvestris*. Pages 643-654 *in* Thimann, K.V., ed. The physiology of forest trees. Ronald Press, New York.

Wareing, P.F. 1953. Experimental induction of male cones in *Pinus sylvestris*. Nature 171: 47.

Wareing, P.F. 1964. Phase change in woody plants. Pages 308-309 *in* Abstr. Tenth Internat. Bot. Congr.

Wareing, P.F.; Nasr, T. 1958. Gravimorphism in trees; effects of gravity on growth, apical dominance and flowering in fruit trees. Nature (London) 182: 379-380.

Webber, J.E. 1983. Developmental sequences and metabolic changes associated with cone enhancement treatments in Douglas-fir: I. Flowering and growth responses to root pruning and gibberellin $(GA_{4/7})$ treatments. British Columbia Forest Serv. Progress Report E.P. 799.02. 66 p.

Webber, J.E.; Ross, S.D.; Pharis, R.P.; Owens, J.N. 1985. Interaction between gibberellin $A_{4/7}$ and root pruning on the reproductive and vegetative process in Douglas-fir. II. Effects on growth. Can. J. Forest Res. 15: 348-353.

Wenger, K.F. 1953. The effect of fertilization and injury on the cone and seed production of loblolly pine seed trees. J. For. 51: 570-573.

Wenger, K.F. 1954. The stimulation of loblolly pine seed trees by preharvest release. J. For. 52: 115-118.

Wheeler, N.C. 1978. Exogenous application of gibberellin and auxin to increase flowering in lodgepole pine seedlings. British Columbia Forest Serv. Rep. EP. 767.02. 21 p.

Wheeler, N.C.; Wample, R.L.; Pharis, R.P. 1980. Promotion of flowering in the Pinacea by gibberellins. IV. Seedlings and sexually mature grafts of lodgepole pine. Plant Physiol. 50: 340-346.

Wheeler, N.C.; Ying, C.C.; Murphy, J.C. 1982. Effect of accelerating growth on flowering in lodgepole pine seedlings and grafts. Can. J. Forest Res. 12: 533-537.

White, G. 1981. Controlled pollination of European black alder. Pages 89-91 *in* Franklin, E.C., ed. Pollen management handbook. USDA Forest Serv. Agric. Handbook No. 587.

White, T.L.; Knopp, J.A. 1978. Conelet abortion and ATP levels in longleaf pine. Can. J. Bot. 56: 680-685.

Whitecross, M.I.; Willing, R.R. 1975. Hybridization of incompatible poplars following solvent treatment of stigmas. Experimentia 31: 651-653.

Whitehead, D.R. 1983. Wind pollination: some ecological and evolutionary perspectives. Pages 97-108 *in* Real, L., ed. Pollination Biology. Academic Press, Inc. N.Y.

Willemse, M.Th.M. 1971a. Morphological and quantitative changes in the population of cell organelles during microsporogenesis of *Pinus sylvestris* L. I. Morphological changes from zygotene until prometaphase I. Acta Bot. Neerl. 20: 261-274.

Willemse, M.Th.M. 1971b. Morphological and quantitative changes in the population of cell organelles during microsporogenesis of *Pinus sylvestris* L. II. Morphological changes from prometaphase I until the tetrad stage. Acta Bot. Neerl. 20: 411-427.

Willemse, M.Th.M. 1971c. Morphological and quantitative changes in the population of cell organelles during microsporogenesis of *Pinus sylvestris* L. III. Morphological changes during the tetrad stage and in the young microspore. A quantitative approach to the changes in the population of cell organelles. Acta Bot. Neerl. 20: 498-523.

Willemse, M.Th.M. 1974. Megagametogenesis and formation of neocytoplasm in *Pinus sylvestris* L. Pages 97-102 *in* Linskens, H.F., ed. Fertilization in higher plants. American Elsevier, New York.

Willemse, M.Th.M.; Linskens, H.F. 1969. Development du microgamétophyte chez le *Pinus sylvestris* entre la meiose et la fécondation. Rev. Cytol. Biol. Végét. 32: 121-128.

Williamson, M.J. 1966. Premature abscissions and white oak acorn crops. Forest Sci. 12: 19-21.

Willing, R.R.; Pryor, L.D. 1976. Interspecific hybridization in poplar. Theor. and Appl. Genet. 47: 141-151.

Willson, M.F.; Burley, N. 1983. Mate choice in plants. Princeton Univ. Press, Princeton, N.J. 251 p.

Winjum, J.K.; Johnson, N.E. 1964. Differences in cone numbers, lengths, and cut-counts in the crowns of young open-grown Douglas-fir. J. For. 62: 389-391.

Winton, L.L. 1964. Phenology of normal and forced microsporogenesis in white and black spruce and their F_1 hybrid. Univ. Minn. School of For. Note 153. 2 p.

Worsely, R.G. 1959. The processing of pollen. Silvae Genet. 8: 143-148.

Wright, J.W. 1953. Notes on flowering and fruiting of northeastern trees. USDA Forest Serv., Res. Paper No. NE-60, 38 p.

Wright, J.W. 1959. Species hybridization in white pines. Forest Sci. 5: 210-222.

Yanagihara, T.; Tochiaki, K.; Arai, K. 1960. On the relation between the harvest of Japanese larch seed and meteorological conditions. J. Jpn. For. Soc. 42: 347-351.

Yocum, H.A. 1971. Releasing shortleaf pines increases cone and seed production. USDA Forest Serv. Res. Note No. SO-125.

Yokoyama, T.; Asakawa, S. 1973. Effect of photoperiod on female strobili formation in *Larix leptolepis* (Sieb. et Zucc.) Gordon. J. Jpn. For. Soc. 55: 388-393.

Yokoyama, T.; Kaneko, T.; Itoo, M.; Yamazaki, S.; Asakawa, S. 1973. The most favorable time and frequency for controlled pollination in *Larix leptolepis* (Seib. et Zucc.) Gord. Jpn. Gov. For. Exper. Stn. Bull. No. 253: 39-53.

Yokoyama, T.; Kaneko, T.; Itoo, M. 1978. The number of pollen grains produced from a male strobilus in *Larix leptolepis* Gord. J. Jpn. For. Soc. 60: 71-73.

Young, E.; Hanover, J.W. 1976. Accelerating maturity in *Picea* seedlings. Acta Hort. 56: 105-114.

Young, L.C.T.; Stanley, R.G. 1963. Incorporation of tritiated nucleosides thymidine, uridine, and cytidine in nuclei of germinating pine pollen. Nucleus 6: 83-90.

Zahner, R.; Stage, A.R. 1966. A procedure for calculating daily moisture stress and its utility in regressions of tree growth on weather. Ecology 47: 64-74.

Zasada, J.C.; Gregory, R.A. 1972. Paper birch seed production in the Tanana Valley, Alaska. USDA Forest Serv. Res. Note No. PNW-177. 7 p.

Zasada, J.C.; Viereck, L.A. 1970. White spruce cone and seed production in Interior Alaska, 1957-68. USDA Forest Serv. Res. Note. No. PNW-129. 11 p.

Zeevart, J.A.D. 1976. Physiology of flower formation. Ann. Rev. Plant Physiol. 27: 321-348.

Zeevart, J.A.D. 1983. Gibberellins and flowering. Pages 333-374 in Crozier, A., ed. The biochemistry and physiology of gibberellins. Praeger, N.Y.

Zenke, U. 1953. Studies on the course of meiosis in Pseudotsuga taxifolia Britton. Silvae Genet. 2: 96-102.

APPENDICES

Appendix 1. Effects of fertilizer on flowering

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
Acer saccharum	ammonium sulfate	April 1936	heavy seed crop 1937	Chandler 1938
Fagus grandiflora and Fagus sylvatica	ammonium nitrate P_2O_5, CaO, K_2O	May 1973, 1974 April 1973	increased m[1] & f	Le Tacon et al. 1977
Juglans nigra	N(urea), P,K	late April	increased nut production	Ponder 1979
Picea abies	N(urea), P,K	not specified	increased in a good flowering year, no effect in poor year	Malkönen 1971
P. abies	N[2],P,K	not specified	no effect	Remrod 1972
P. glauca	ammonium nitrate	late May, early June	increased flowering	Holst 1959
	N(urea),P,K	late May, early June	slightly increased flowering	
Pinus banksiana	ammonium nitrate	early May	increased f (more than early June), decreased m	Holst 1971
		early June	increased f	
		early July	no effect	
		early August	decreased f	
P. echinata	N,P,K	April for 2 years	increased seed production	Brinkman 1962
P. elliottii	N,P,K	mid-April	increased f	Hoekstra and Mergen 1957
P. elliottii	ammonium nitrate	early April, early June	increased f	Barnes and Bengston 1968

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[1] m refers to male flowers and pollen cones, f to female flowers and seed cones.
[2] Where form of nitrogen is not indicated, it has not been specified.

Appendix 1 (cont.)

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
P. elliottii	uramite(=urea) superphosphate	April	no effect	Benston 1969
P. elliottii	ammonium nitrate	spring	increased f	Schultz 1971
P. lambertiana	ammonium phcsphate	spring, for 3 years	increased cone production	Schubert 1956
P. monticola	ammonium	April 30 late-May	increased f on 11-yr old trees no effect on 11-yr old or mature trees	Barnes and Bingham 1963
P. monticola	N,P,K or ammonium nitrate	Aug. 1960, 1961	increased seeds/tree 1961 only	Barnes 1969
P. palustris	N,P,K	Feb. 1949, 1951	no effect for 1st 2 yrs, cone production increased in 3rd and 4th yr	Allen 1953
P. palustris	N,P,K	each spring for 5 yrs.	no effect on cone production	Crocker 1964
P. palustris	N,P,K	March to May	time of application had no effect; effect of fertilizer was dependent on rainfall: wet spring increased flowering dry spring decreased flowering	Shoulders 1967, 1968
P. palustris	N,P,K	July 21 - Aug. 1	increased cone production	McLemore 1975
P. pinaster	N,P,K	May	increased flowering	Illy 1963

Appendix 1 (cont.)

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
P. ponderosa	urea, ammonium phosphate	late fall, early winter (on snow)	increased cone production	Heidman et al. 1979
P. radiata	superphosphate	not specified	increased cone production	Anonymous 1957a
P. radiata	urea, ammonium nitrate	not specified	no effect	Sweet and Hong 1978
P. resinosa	ammonium nitrate	late May, early June	June treatments increased f	Holst 1959
P. resinosa	ammonium nitrate	May 22 (bud break)	increased flowering	Cayford and Jarvis 1967
P. resinosa	N(urea),P,K	early May	increased flowering	Cooley 1970
P. resinosa	ammonium nitrate	May 24 or July 24	increased flowering 50% in upper 4 whorls 1st year, decreased f 16% 2 yrs. After application	Holst 1971
		July 28	increased f 24% in upper 2 whorls 1st yr, decreased f 13% 2nd yr	
		Aug. 20 or Sept. 19	no effect 1st yr, increased f in upper 2 whorls 66%, and 121% in whorls 3 and 4, 2nd yr	
P. strobus	N,P,K	spring	increased flowering	Hocker 1962
P. strobus	ammonium nitrate	April, June & July	increased female flowering on 22- but not 13-14-year old trees	Stephens 1964
		April, June & August	no effect	

Appendix 1 (cont.)

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
P. sylvestris	N,P,K	late May	increased f	Nanson 1965
P. sylvestris	N,P,K boron and manganese (foliar spray)	not specified	increased f increased seed production	Enescu and Giurgiu 1968
P. sylvestris	N,P,K	May, for 2 years	increased cone and conelet production	Hattemer et al. 1977
P. taeda	N,P,K	April	increased cone production of 25-yr old trees in poor-flowering yr, no effect on 40-yr old trees	Wenger 1953
P. taeda	N,P,K	May	increased number of cones per tree	Schmidtling 1971
P. taeda	N,P,K	April, May, for 5 years	no effect (fertile site)	Pitcher 1972
P. taeda	ammonium nitrate	early May, Aug. & late Nov.	increased f	Greenwood 1977
P. virginiana	N,P,K	March	increased m & f	Bramlett and Belanger 1976
Southern pines (species not given)	ammonium nitrate	late July	increased flowering and cone production, greater effect when applied in conjunction with water stress of subsoiling	Sprague et al. 1979
Pseudotsuga menziesii	N,P	not specified	increased cone production sixfold increased number of filled seed 20%	Anonymous 1957b
P. menziesii	nitrate	July or Sept., then May for 2 yrs	increased number of flowers and per cent of trees flowering	Steinbrenner et al. 1960

Appendix 1 (cont.)

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
P. menziesii	nitrates	spring	increased f 1st year	Ebell 1962
	ammonium	spring	increased f 2nd year	
	P,K	spring	no effect	
P. menziesii	nitrate	mid-April late May mid-June	mid-June ineffective, others increased cone production and decreased aborted buds, late May best	Ebell 1967a
P. menziesii	ammonium nitrate	May 8 and June 2	increased m when applied in conjunction with water stress	Melchior 1968
P. menziesii	ammonium nitrate	May	increased cone production on wild trees, no effect on plantation trees	Smith et al. 1968
P. menziesii	nitrate	May 19	increased f	Ebell and McMullan 1970
	ammonium	May 19	no effect	
P. menziesii	nitrate	Apr. 14, May 21, June 11	increased cone production, May was best.	Ebell 1972a
	ammonium	Apr. 14, May 21, June 11	no effect	
P. menziesii	ammonium nitrate	May	increased cone production	Ebell 1972b
P. menziesii	nitrate	April 8	increased f, no effect when applied with water stress	Cade and Jackson 1976
P. menziesii	N,P,K	early spring	increased m & f 1978	Enescu et al. 1978

Appendix 2. Effects of girdling, banding, and strangulation on flowering

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
Castanea henryi seedlings	girdling	May 27	abundant m[1] flowering	Graves 1958
Cryptomeria japonica	girdling	June/July	increased flowering[2] the yr of treatment	Hashizume 1970
	banding	July	increased flowering the yr after treatment	
Fraxinus nigra	girdling	mid-May	increased flowering	Pond 1936
	banding	mid-May	increased flowering	
Larix decidua	girdling	up to the end of May	increased m & f	Melchior 1960
L. leptolepis	trunk girdling	May 30, June 18, & 21	1-year treatment increased the number of m-bearing trees 2-year treatment did not increase f	Hashizume 1967
	branch girdling	June 18	no effect, some f formed but abscised	
	branch strangulation	June 15	continuous strangulation over several yrs increased f	
L. leptolepis	strangulation	June	increased f and the number of flowering grafts	Heitmuller and Melchior 1960
L. leptolepis	girdling	May	increased m & f	Melchior 1961a
	strangulation	May	no effect	
L. leptolepis	girdling	up to the end of May	increased m & f initiation that year	Melchior 1960
		late June-July	increased flower initiation the following year	

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[1] m refers to male flowers and pollen cones, f to female flowers and seed cones.

[2] Unless specified, the author has not mentioned if flowering refers to males or females.

Appendix 2 (cont.)

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
Picea glauca	girdling	early June	increased flowering	Holst 1959
	strangulation	early June	no effect	
Pinus echinata	girdling	winter	tripled cone production 3rd year after treatment	Bower and Smith 1961
P. elliottii	girdling	not specified	increased f	Hoekstra and Mergen 1957
	strangulation	not specified	increased f but less effective than girdling	
P. laricio	girdling	Sept., Jan. or May	May treatment most effective, Jan. least effective	Faulkner 1966
P. palustris	girdling	mid-May	increased the number of m branches	Varnell 1970
P. ponderosa	girdling	May	increased cone production[3]	Shearer and Schmidt 1970
P. sylvestris	gridling	April	increased m & f, but damaged or killed trees	Faulkner 1966
P. strobus	branch girdling	April, June, or early & late July	late July treatment increased flowering on treated branch	Stephens 1964
	branch strangulation	same dates	no effect	
P. taeda	girdling	early April	increased flowering but damaged trees	Bilan 1960
	banding	early April	decreased flowering	
P. taeda	girdling	mid-Feb.	increased f	Hansbrough and Merrifield 1963

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[3] Author does not mention whether this is due to increased flower initiation.

Appendix 2 (cont.)

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
Pseudotsuga menziesii	girdling	May, June, July	no effect	Melchior 1968
	strangulation	late June	increased m & f the 2nd yr after treatment	
P. menziesii	girdling	late Aug. 1957	increased f in 1959	Ebell 1971
		mid-May 1958	increased f in 1959	
P. menziesii	girdling	mid-April	increased f (best when applied with nitrate fertilizer)	Cade and Jackson 1976
P. menziesii	girdling	mid-April	increased f	Cade and Hsin 1977

Appendix 3. Effects of root pruning (RP) and other root treatments on flowering

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
Larix leptolepis	RP	not specified	no effect	Heitmüller and Melchior 1960
Picea glauca	RP	April 30	increased flowering when applied with ammonium nitrate fertilizer	Holst 1959
Pinus elliottii	RP	end of March	increased f[1]	Hoekstra and Mergen 1957
P. elliottii	disking	4 times/year 1960-65 2 times/year 1965-69	increased m when irrigation also applied	Schultz 1971
P. radiata	confined roots	2 years	increased m	Sweet and Will 1965
P. resinosa	transplanting, then confined roots	April	high cone production while roots confined	Quirk 1973
P. strobus	RP	July	increased f	Stephens 1964
P. taeda	subsoiling	July 30-31	increased f	Gregory and Davey 1977
Southern pines (species not given)	subsoiling	late June - early Aug.	increased cone production	Sprague et al. 1979
Pseudotsuga menziesii	RP	late May, early June	increased m	Melchior 1968
P. menziesii	lifting and replanting	June 30 - July 1	increased f	Silen 1973

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[1] m refers to male flowers and pollen cones, f to female flowers and seed cones.

(Note: See Appendix 8 for RP effects when applied as adjunct treatment to gibberellins).

Appendix 4. Effects of moisture stress and irrigation on flowering

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
Fagus	water stress, the year of floral initiation	June 17 - August 12	increased flowering	Holmsgaard and Olson 1960, 1961
Pinus elliottii	irrigation	all year	increased m[1]	Barnes and Bengston 1968
P. elliottii	irrigation	all year	increased m	Bengston 1969
P. elliottii	irrigation	all year	increased m when applied with disking	Schultz 1971
P. palustris	irrigation	April to Oct.	no effect on cone production	Crocker 1964
P. taeda	irrigation and water stress	various times	late summer irrigation decreased m, April-June irrigation plus July-Sept. drought increased conelet crop	Dewers, and Moehring 1970
Southern pines	irrigation	spring and early summer, and after initiation	increased cone production if ammonium nitrate fertilizer also applied	Sprague et al. 1979
Pseudotsuga menziesii	water stress	not specified	increased male flowering when applied with ammonium nitrate fertilizer; no effect alone	Melchior 1968
P. menziesii	water stress	bracketing time of vegetative bud break	increased m and f	Ebel 1967a,b
P. menziesii	water stress	April 28 - July 1	decreased flowering when applied alone, increased f when applied with nitrate fertilizer	Cade and Jackson 1976

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[1] m refers to male flowers and pollen cones, f to female flowers and seed cones.

(Note: See Appendix 8 for water stress effects when applied as adjunct treatment to gibberellins).

Appendix 5. Effects of thinning, spacing, and release on flowering

SPECIES	EFFECT ON FLOWERING	REFERENCE
Araucaria cunninghamia	increased cone production - 1and 2 yrs after treatment	Florence and McWilliam 1956
Juglans nigra	increased nut production 2 yrs after treatment	Ponder 1979
Larix leptolepis	increased seed production, enhanced by fertilization	Asakawa and Fujita 1966
Pinus densiflora	increased seed production, enhanced by fertilization	Asakawa and Fujita 1966
P. echinata	increased seed production	Phares and Rogers 1962; Yocum 1971; Dorman 1976
P. elliottii	increased cone production 1 and 2 yrs after treatment	Florence and McWilliam 1956
P. palustris	increased cone production 3 and 4 yrs after treatment	Allen 1953
P. ponderosa	increased cone production 2 and 4 yrs after treatment when fertilizer applied	Heidmann et al. 1979
P. radiata	increased seed production/acre for 5 years after treatment	Eldridge 1966
P. resinosa	increased number of cones per tree when checked 10 yrs after thinning	Godman 1962
P. resinosa	increased [1]m and flowering 3 and 6 yrs after thinning	Cooley 1970
P. taeda	increased cone production the 3rd yr after release if treatment occurred before June-July	Wenger 1954
P. taeda	increased seed production and percent sound seed	Allen and Trousdell 1961

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[1] m refers to male flowers and pollen cones, f to female flowers and seed cones.

Appendix 5 (cont.)

SPECIES	EFFECT ON FLOWERING	REFERENCE
P. taeda	increased cone and seed production 3 and 4 yrs after treatment	Bilan 1960
P. taeda	increased cone production 1 and 2 yrs after treatment	Florence and McWilliam 1956
Pseudotsuga menziesii	in good yrs thinning greatly increased seed production, no effect in poor seed yrs	Reukema 1961

Appendix 6. Effects of light treatments on flowering

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
Betula sp. seedlings	1) continuous illumination 15-25°C	begun early June	increased m[1]	Longman and Wareing 1959
	2) short days (SD) (9 hrs) 0-5°C for 6 wks then long (LD)		no effect on flowering	
Cryptomeria japonica, *Chamaecyparis obtusa* seedlings	tested effects of temperature (T) and light after GA treatment	summer or autumn	*C. japonica*: high T, LD increased m; low T, SD increased f; blue and far red light increased m; red light increased f *C. obtusa*: high alternate T (30°C-20°), LD with high light intensity for more than 16 hrs and red light all increased m & f	Nagao and Sasaki 1981
Chamaecyparis obtusa	as above with following conditions: Temp (°C): day, night 30° 25° 25° 20° 20° 15° Light: normal (360-760 nm), blue (360-560 & 700-760 nm), yellow (450-760 nm), red (560-760 nm)	not specified	most m & f under red & yellow light, 30° day, 25° night blue light gave less m & f than normal light	Nagao 1983a

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[1] m refers to male flowers and pollen cones, f to female flowers and seed cones.

Appendix 5 (cont.)

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
Larix leptolepis	photoperiod (PP)	not specified	f formed under 12, 14, 15, and 16 hr PP, but not under 10, 18, 20 hr PP	Yokoyama and Asakawa 1973
Picea sp.	24 hr. PP optimum moisture, temp. and nutrients	grown from seed for the 1st year of growth	flowering occurs at age 4 yrs. from seed	Young and Hanover 1976
P. glauca	night-interrupted exposures of red light	various times throughout the growing season	decreased f	Durzan and Campbell 1979; Durzan et al. 1979
Pinus sp.	change of photoperiod by moving to different latitude	not applicable	no effect on flowering	Mirov 1956
P. attenuata	interrupted nights	March 21 to June 21, June 22 to Sept. 21	no effect on the number of trees flowering	Lanner 1963
P. contorta	24 hr PP for 6 months	Jan. to mid-June (seed sown in Jan.)	increased per cent trees flowering, and the number of m & f per flowering tree at 5 yrs from seed	Wheeler et al. 1982
P. contorta	short days	not applicable	increased f if grown outside, but no f produced if grown in greenhouse	Longman 1961

Appendix 6 (cont.)

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
P. taeda	out-of-phase-dormancy: 20 hr PP in heated greenhouse Oct.-Feb., then T lowered and PP shortened mid-Feb.		increased m & f	Greenwood 1978a
Salix petandra	various PP	not applicable	cuttings from southern ecotypes SD (12 hr) resulted in best flower bud development, most flower buds per plant, and longest inflorescence primordia cuttings from northern ecotypes. Strong T-PP interaction in high T, short PP inhibited flower bud formation, otherwise effect of PP on flowering not clear.	Junttila 1980

Appendix 7. Effects of miscellaneous treatments on flowering

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
A. Pruning, topping				
Pinus elliottii	branch pruning	March-April	decreased number of branches in 1st yr	Varnell 1969
P. monticola	stem breakage	(natural causes)	increased m[1] and f	Coffen and Bordelon 1981
P. sylvestris	removing terminal bud	Jan.	increased m	Wareing 1953
P. sylvestris	pruning	various times	no effect on f pruning old shoots in Feb., Mar., and Apr. increased m pruning shoots at later dates had no effect on m	Melchior and Heitmüller 1961
Pseudotsuga menziesii	annual pruning to yearly growth for 6 yrs	July and Sept.	number of cones per foot of tree height was the same as control	Copes 1973
B. Polythene covers, plastic tents, plastic greenhouses				
Betula	plastic greenhouse	spring	applied in conjunction with high CO_2, resulted in good seed crop from 1-3 yr old seedlings	Lepisto 1973
Picea abies	plastic tents	put on before pollination in June, removed in July	increased seed quality, stimulated m and f	Remrod 1972

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[1] m refers to male flowers and pollen cones, f to female flowers and seed cones.

Appendix 7 (cont.)

SPECIES	TREATMENT	DATE	EFFECT ON FLOWERING	REFERENCE
P. abies	polythene covers	June 3-July 5 June 3-July 19	increased number of m per graft, but not the number of flowering grafts	Chalupka and Giertych 1977
P. sitchensis	polythene house	May-August	increased m and f	Tompsett and Fletcher 1977
Pinus sylvestris	plastic film around trees 1 m above ground	not specified	no effect on flowering	Bergman and Kardell 1975
C. Gravity				
Larix	inverting branches	March & April	flower buds developed on lower (previously upper) side of branches from previously vegetative buds (lower side of branch is where flower buds normally develop in *Larix*)	Longman 1961
L. leptolepis	bending top of crown	not specified	increased f	Heitmüller and Melchior 1960
L. leptolepis	bending top of crown	not specified	decreased f	Melchior 1961a
L. leptolepis	tying branches into horizontal and downward positions	March 22 - April 26	increased m, especially in good flowering yr, and downward-pointing branches	Longman et al. 1965; Longman and Wareing 1958

Appendix 7 (cont.)

SPECIES	TREATMENT	EFFECT ON FLOWERING	REFERENCE
D. Grafting			
Picea abies	varying rootstock	no significant effects on flowering	Krusche and Melchior 1978
Pinus	several species of rootstock and scions	all combinations produced m & f flowers, female cone production was best on combinations having longest needles	Ahlgren 1972
P. monticola	grafting seedling scions into crown of (a) mature trees, (b) 10-15 yr old, near maturity	no flowering on scions in either case	Barnes and Bingham 1963
P. sylvestris	grafting: (a) juvenile scion into adult tree, (b) adult scion, juvenile stock	flowering in 1 case only, juvenile scion into adult 25 yr old tree, 2 yrs after grafting	Simak 1979
P. taeda	grafting scions from 1 yr seedlings to crowns of adults	m produced on 50% of surviving grafts 2 yrs after grafting, female flowers on 20% only	Creenwood and Gladstone 1978
E. Cover crop			
Pinus elliottii	legume cover crop	increased f on irrigated plots; decreased f on non-irrigated plots	Barnes and Bengston 1968
P. elliottii	legume cover crop	increased m with irrigation and no fertilizer	Schultz 1971

Appendix 8. Effects of growth regulators (GR) on flowering

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
Larix				
Larix decidua	GA_3, NAA, TIBA, 6BA by IV[2] last week of April & May (before differentiation)		NAA increased m[1] but inhibited f, TIBA increased f but inhibited m, GA_3 no effect	Hall 1977
L. leptolepis	NAA, CCC (GA inhibitor), BCB, B995, ethrel, BOH, IAA, TIBA	girdling, shoot pinching, defoliation, heavy root-pruning (RP) transplanting	no real success, high mortality, no statistical analysis	Mikami et al. 1979
L. leptolepis Abies homolepis grafts	$GA_{4/7}$, GA_4, GA_3 directly into cuts in branch $GA_{4/7}$ foliar spray (treatment only applied at the supposed time of floral differentiation)	girdling	girdling increased m in Larix,	Katsuta et al. 1981
L. leptolepis	GA_3 + $GA_{4/7}$, GA_3 + $GA_{4/7}$, $GA_{4/7}$, and $GA_{4/7}$ + NAA by IV, foliar spray	girdling plastic mulching	$GA_{4/7}$ increased m & f, mulching enhanced the effect May & June application was best IV more effective than foliar spray	Bonnet-Masimbert 1982
Picea				
Picea abies	foliar spray 3-year seedlings 1) GA_3, IAA or kinetin (KI) 3 times at weekly intervals, in the spring		3-year seedlings no effect	Bleymuller 1976

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[1] m refers to male flowersand pollen cones, f to female flowers and seed cones.

[2] IV refers to GR applied with intervenous apparatus feeding into drilled hole.

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
	2) then: chlormequat (CCC) or malic hydrazide (MH, all possible combinations of 1 & 2			
	16-year-old 1) GA$_3$, IAA, or KI, once at bud burst then 2) CCC or MH (4 and 8 weeks later) all possible combinations of 1 & 2		16-year-old GA$_3$ + CCC increased f, decreased m, KI + MH increased f, significant clonal differences	
P. abies	GA$_3$ + CCC, GA$_3$ + MH, IAA + CCC, KI + MH foliar spray		all treatments inhibited m, GA + CCC, KI + MH, IAA + CCC increased f	Bleymuller 1978
P. abies 1979 grafts	GA$_3$ 5/24, 5/28, 6/1,		GA$_3$ increased m, CCC alone,	Chalupka 1981
	CCC mid-June, foliar spray		no effect, GA$_3$ + CCC increased m (synergistic)	
P. abies grafts	GA$_{4/7}$ + GA$_3$ + NAA; foliar spray, 4 applications 5/25-6/16 timing experiment - 1 application between 5/20 & 6/26	plastic greenhouse, field	field, no effect greenhouse + hormone 5/31 increased f 5/31 & 6/1 (when shoot elongation was 50 per cent total), increased m, 5/20 (start of shoot elongation), no increase in m	Luukkanen 1979
P. abies mature grafts	1975 GA$_3$, GA$_{4/7}$, GA$_3$ + GA$_9$, GA$_{4/7}$ + GA$_9$, and GA$_3$ + GA$_{4/7}$ + GA$_9$		1975 GA$_{4/7}$ + GA$_9$ increased f most, GA$_{4/7}$ alone increased f, but was enhanced by GA$_9$	Dunberg 1980

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
	1977 GA_4, $GA_{4/7}$, GA_9, GA_4 + GA_9, $GA_{4/7}$ + GA_9, EtOH control topical, 3 times: 6/4 (start of shoot elongation), 6/24, 7/8 (end of shoot elongation)		1977 Statistical results don't agree with 1975 results, due to EtOH causing increased f. The only significant difference was increased f by GA_9	
P. abies grafts	1) GA_3 - foliar spray 5/12, 5/22, 5/30 2) GA_3, NAA, GA_3 + NAA - foliar spray, 5/19, 5/26, 6/4, 6/9	± polythene covers 6/18 - 7/24	GA_3 + polythene increased m (additive, no synergism) NAA - slight effect only	Chalupka 1981
P. sitchensis 5 & 10 yr. grafts	5 yr. grafts GA_4 ± KN, GA_4 ± DPU, GA_4 + GA_5 + GA_3 + DPU, $GA_{4/7}$ - topical, weekly, 12 times July to mid-Sept.	Phosphon D ABA, BA, NAA Polythene house	$GA_{4/7}$, $GA_{4/7}$ + GA_5, and GA_9 all increased m & f in polythene house Phosphon D and ABA reversed GA effect.	Tompsett 1977, 1978b
	10 yr. grafts $GA_{4/7}$ ± GA_3, $GA_{4/7}$ ± GA_4, $GA_{4/7}$ ± GA_5, hyposyringe near base of branch under bark, 2 applications 7/6, 8/8	field	GA's all increased m & f BA enhanced GA effect NAA decreased f, increased m	
P. sitchensis 2 & 11 yr. grafts	2 yr. grafts $GA_{4/7}$ + GA_3, topical	various GA concentration & time of application polythene house	Better flowering than 11 yr. grafts in field, May and June applications gave best flowering, flowering increased with increasing GA application up to 1.97 mg/ℓ.	Tompsett and Fletcher 1979

Appendix 8 (cont)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
	11 yr. grafts $GA_{4/7}$ + GA_3, hyposyringe under bark		4 mg/l GA in June increased m & f	
P. abies 5 yr. grafts	$GA_{4/7}$ - hole bored in main stem, once in May, June and July	polythene house + heat + H_2O stress (cool, wet; cool, dry; hot, wet; hot, dry)	heat ± GA increased m & f, H_2O stress ± GA increased m & f, GA did not make a significant difference in m flowering but significantly increased f.	Philipson 1983
P. mariana P. glauca seedlings	P. mariana: GA_3 foliar spray, 3 times/week for 3 months then 2 times/week P. glauca + P. mariana: GA_3 to rhizosphere	growth chambers 21°C, photoperiod of 8, 16 or 24 hours	No m or f foliar spray increased apical growth, decreased lateral branching, root treatment increased apical growth	Fraser 1969
P. mariana P. glauca	$GA_{4/7}$ for 8 weeks time and method of application unspecified	RP	P. mariana GA increased f (X4) and m (X10) P. glauca GA increased f (X6) and m (X2) RP increased f (X5) and m (X1.5)	Ho 1982
P. glauca 8 & 9 yrs.	$GA_{4/7}$ foliar spray, weekly 6/3 - 7/1 (to bracket time of differentiation), to whorls 3 & 4 (strictly) male & transitional zones) whorl 2 = control		transitional zone branches: increased f (X6.2) and m (X2.4) male zone branches: induced f, increased m (X6)	Marquard and Hanover 1984

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
Pinus				
Pinus banksiana seedlings	$GA_{4/7}$ ± NAA foliar spray	accelerated growth in greenhouse prior to outplanting, seeds sown in Oct., Jan., March	March sowing - no flowering, other sowings - increased f with either GA treatment	Cecich 1981
P. banksiana	$GA_{4/7}$ weekly at dawn 200, 400, 600 mg/ℓ	Timing: 3 sets	GA conc. 200 400 600	Cecich 1983
		1) a) April 28 - May 6 early shoot elongation	- - -	
		b) June 2 - 30 late shoot elongation	- m m	
		c) July 7 - Aug. 4 early bud development	m m m	
		d) Aug. 11 - Sept. 9 late bud development	- - -	
		2) a) April 28 - June 30 shoot elongation	- m -	
		b) July 7 - Sept. 9 bud development	- m mf best	
		3) a) April 28 - Sept. 9 growing season	m mf mf	
P. caribaea	GA_3	photoperiod (LD, SD, normal - time periods not specified)	no effect	Rawal and Agrawal 1982
P. contorta	GA_3, $GA_{4/7}$, GA_9 topical 1) biweekly June & July (covering the time of m cone initiation) directly to bud apex of each of 3 lateral shoots subtending the terminal shoots	± girdle	1) no effect on m	Pharis et al. 1975

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
	2) GA_3 and/or $GA_{4/7}$ as above, except during Aug. & Sept. (covering the time of f cone initiation) to the terminal apex		2) GA_3 + $GA_{4/7}$ increased f, synergistic	
P. contorta	$GA_{4/7}$ + NAA foliar spray		increased f on seedlings which had already expressed flowering potential	Wheeler 1978
P. contorta 6 yr. seedlings grafts	6 yr. seedlings: NAA, IAA, $GA_{4/7}$ ± NAA, GA_3, $GA_{4/7}$, GA_3 + $GA_{4/7}$ grafts: $GA_{4/7}$, $GA_{4/7}$ ± NAA (NAA - Aug. & Sept.) topical and foliar spray June - Sept.	girdling NO_3 fertilizer	girdling + GAs increased m, any of the GA applications increased f	Wheeler et al. 1980
P. contorta rooted cuttings	$GA_{4/7}$ small hole drilled in base of plant 6/8, 6/17	Photoperiod (SD = 10hr, LD = 19.5hr.) Temperature (°C) cool=15° day, 8°night warm=22°day, 15°night	cool increased m, SD better than LD for f, GA gave no significant effect	Longman 1982
P. elliottii P. palustris P. taeda	$GA_{4/7}$ topical or foliar spray, 2 or 3 biweekly treatments May - Aug.		only m induced in all 3 species Best treatments: P. elliottii topical 7/9-8/16 or foliar spray (GA + NAA) 7/26-8/23 P. taeda foliar spray (GA + NAA) 7/01-7/29	Hare 1984

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
			P. palustris topical 6 weeks	
P. palustris	$GA_{4/7}$ + NAA topical 6/1-8/10	NH_4NO_3 fertilizer, girdling, cultivation	GA + NAA, GA + NAA + fert, and GA + NAA + fert + girdling all increased m & f.	Hare et al. 1979
P. radiata	GA_3, $GA_{4/7}$, GA_9, ± NAA	various GA concentrations	no significant difference, $GA_{4/7}$ + NAA best	Sweet and Hong 1975
P. radiata	$GA_{4/7}$ + NAA topical for 6 weeks	urea fertilizer, girdling	increased f 36% (significant at 15% level)	Carson et al. 1977
P. radiata	$GA_{4/7}$ + NA topical, twice during long shoot differentiation, when LSB recognizable, but not differentiated (mid-Feb.)		increased f	Sweet 1979
P. radiata	$GA_{4/7}$ topical 2/14, 2/19 1978 2/8, 2/13, 2/22 1979	studied photoassimilate partitioning	increased f	Ross et al. 1984
P. sylvestris mature grafts	$GA_{4/7}$, 3 methods of application 1) in lanoline into longitudinal 4-5 cm slit in stem 5/12 2) foliar spray 5/6, 5/13, 5/20 3) foliar dip 5/6, 5/13, 5/20 ±CCC dip & spray 6/18, 6/25, 7/2 lanoline 7/3	± girdling	CCC no measurable effect $GA_{4/7}$ increased m not f girdling increased m & f, GA + girdling not synergistic for m additive only	Chalupka 1978

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
P. sylvestris grafts	$GA_{4/7}$, GA_3, GA_3 + $GA_{4/7}$ foliar spray, late May & June, mid & late July, mid-Aug.		GA_3 negative effect on m $GA_{4/7}$ increased f	Chalupka 1980
P. sylvestris 12 yr. grafts Picea abies 6 yr. grafts	$GA_{4/7}$ ± NAA, $GA_{4/7}$ + GA_3 foliar spray every 10-14 days 5/24 - 7/16, 5 times total		Pinus all treatments increased m & f, no significant difference between GA treatments Picea no significant increase in flowering	Luukkanen and Johansson 1980a
P. sylvestris	as above, plus GA_9 in 1977 only and GA_3, every 10-20 days, 5 times in 1976, 3 times in 1977		GA_3 and GA_9 not effective	Luukkanen and and Johansson 1980b
P. sylvestris grafts	$GA_{4/7}$, NAA, $GA_{4/7}$ + NAA foliar spray 5/25, 5/29 6/7, 6/13	± polythene wrapped around branch	NAA only very slight effects, GA increased m, decreased f, no synergism between GA & polythene, additive only	Chalupka 1981
P. sylvestris 9 yr. grafts	GA_4, GA_7, $GA_{4/7}$, control, foliar spray to elongating shoots 6/12, 6/19, 6/26, 7/17		increased f - GA_4 best, GA_7 worst (small sample size, no statistics).	Luukkanen 1981
P. taeda P. elliottii	$GA_{4/7}$, NAA, Ethephon, CCC, arginine topical to branch tips, biweekly 6/1 - 8/10	girdling, itaconic acid bud treatment, sucrose foliar spray, NH_4NO_3 fertilizer	GA + NAA + girdling increased m most, NAA + arginine increased m least, f not affected	Hare 1979

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
P. taeda mature grafts immature grafts	mature grafts GA_3, GA_5, $GA_{4/7}$, EtOH		mature grafts: EtOH control increased f, making GA increase not significant; effect of $GA_{4/7}$ was equivalent to GA_3 at 500 mg/ℓ	Ross and Greenwood 1979
	immature grafts $GA_{4/7}$, GA_3, $GA_{4/7}$ + GA_3 topical, 5/20 - mid-Sept.	±girdle	immature grafts: $GA_{4/7}$ increased f, the effect of $Ga_{4/7}$ was greater than GA_3	
P. taeda	$GA_{4/7}$ topical, biweekly mid-June to late Sept.	H_2O stress out-of-phase dormancy (OPD)	OPD increased m & f GA + H_2O stress increased f	Greenwood 1981
P. taeda 3 yr. graft	GA_4, GA_7, $GA_{4/7}$, GA_3 1) topical to stem at base of bud 2) foliar spray 3) IV	H_2O stress 7/1 - 9/30	topical GA_4, GA_7 & $GA_{4/7}$ increased f, best time of application was biweekly 6/18 - 10/22	Greenwood 1982
P. taeda	GA_3, $GA_{4/7}$	pot culture, H_2O stress, girdling, RP	RP decreased m, pot culture + $GA_{4/7}$ + H_2O increased f, RP increased f	Greenwood 1978b
P. thunbergii P. densiflora	$GA_{4/7}$, GA_3, CMC small incisions at branch base in lower branches of crown, 1 application between late June & late July		$GA_{4/7}$, GA_3 increased m of both species and increased f of P. densiflora, no effect on f of P. thunbergii	Kanekawa and Katsuta 1982; Katsuta 1981

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
Pseudotsuga				
Pseudotsuga menziesii grafts	$GA_{4/7}$, GA_5, GA_9, ± BA, ±TIBA GA topical BA topical TIBA foliar spray biweekly late March to early June, 2nd & 3rd whorl branches	± gridling	$GA_{4/7}$ at 40 mg/branch better than at 400 mg/branch for m $GA_{4/7}$ at 400 mg/branch best for f gridling + GA decreased seed set, GA alone increased seed set 6X over control	Ross 1975
P. menziesii 2 yr. grafts 4 yr. seedlings	GA_3, GA_5, GA_9, $GA_{4/7}$, NAA topical, early April to mid-June		$GA_{4/7}$ increased f on grafts, GA_3 less effective, GA_5 & NAA both increased m on grafts, but not significantly	Ross 1976
P. menziesii 4 yr. grafts	$GA_{4/7}$ ± (GA_9, GA_5, BA or TIBA) bark incision, biweekly late March to late June	branch girdling	$GA_{4/7}$ ± (GA_5 or GA_9) increased m & f, but worked well only on clones with a history of flowering, girdling enhanced the effect.	Ross and Pharis 1976a, b
P. menziesii 2 yr. grafts 4 yr. seedlings	$GA_{4/7}$ IV	partial girdling, H_2O stress, NH_4 or NO_3 fertilizer, pot culture	grafts $GA_{4/7}$ increased m & f, pot culture caused profuse flowering seedlings $GA_{4/7}$ - H_2O stress + NO_3 increased m, $GA_{4/7}$ + girdle increased f	Ross 1978
P. menziesii grafts seedlings	$GA_{4/7}$, NAA, $GA_{4/7}$ + NAA, topical to leaf traces of freshly removed needles just below bud, biweekly	± girdling	no significant effect on m, $GA_{4/7}$ ± NA increased f	Puritch et al. 1979

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
	mid-late April to mid-July			
P. menziesii 4 yr. grafts 6 yr. seedlings	grafts $GA_{4/7}$, GA_5, GA_9 bark incision, ± BA ± TIBA foliar spray seedlings GA_3, $GA_{4/7}$, GA_5, GA_9 ± NAA bark incision, biweekly late March to late June	girdling	no effect on m grafts 400 ±g $GA_{4/7}$ increased seed cones and filled seed, girdling decreased filled seed seedlings 400 ±g $GA_{4/7}$ increased seed cones and filled seed, GA_9 + $GA_{4/7}$ gave a significant increase over $GA_{4/7}$ alone	Ross et al. 1980
P. menziesii 6 yr. seedlings	GA_3, GA_5, GA_9, $GA_{4/7}$, ± NAA topical to bark surface, 8 treatments, 4/12 - 6/28		increased m with $GA_{4/7}$ alone and $GA_{4/7}$ + high concentrations of NAA, increased f with $GA_{4/7}$ + GA_9 and $GA_{4/7}$ + low concentrations of NAA	Pharis et al. 1980
P. menziesii grafts	$GA_{4/7}$, ABA, arginine-HCl, control, crude bud extracts of a good flowering tree, extract partially purified (containing auxins, GAs, ABA, etc.), purified GA extract - IV		pure $GA_{4/7}$ increased m & f crude extract increased m	McMullan 1980
P. menziesii seedlings grafts	$GA_{4/7}$ + NAA IV, 5/10 (took 3 weeks to absorb)	1) well irrigated 2) medium H_2O stress 3) hypoxy (flooding roots with stagnant H_2O)	increased m with GA + NAA + 2, GA + NAA + 3, GA + NAA + 3 + 4, increased f with GA + NAA + 2 + 4	Bonnet-Masimbert 1982

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
		4) polythene house concentration and timing of GA		
P. menziesii 2 & 4 yr. rooted cuttings	rooted cuttings various $GA_{4/7}$ concentrations, IV 12 weeks from bud-burst in May		rooted cuttings 2 yr. 25 & 100mg/ℓ 100mg/ℓ gave more f than 25mg/ℓ but may be suboptimal. 4 yr. 50, 100, 200, 400, 800 mg/ℓ 50mg/ℓ was best for f and vegetative growth	Ross 1983a
5 & 6 yr. mature grafts	grafts various times of $GA_{4/7}$ application: 1) pre-bud swell 2/22-4/18 2) bud-burst and after 4/18 - 6/27 3) post bud-burst until the end of shoot elongation 5/30 - 8/8		grafts period 2 gave most f	
P. menziesii grafts seedlings	$Ga_{4/7}$, IV, 9 weeks ending 6/6	RP	m induced in grafts only, f induced in grafts and seedlings, RP alone gave better results than GA alone, RP + GA was synergistic	Ross et al. 1985
Tsuga				
Tsuga heterophylla 3 yr seedlings	$GA_{4/7}$, foliar spray, weekly 5/23 - 6/25	plastic pots, greenhouse, fertilized every 2 weeks 1) 5g $Ca[No_3]_2$ + H_2O stress 2) 10g $Ca[No_3]_2$ + no stress	GA + H_2O stress 20°C increased m & f weak 25°C increased m & f best 30°C increased m & f	Pollard and Portlock 1981a

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
		daytime temperature 20, 25, 30°C		
T. heterophylla mature	$GA_{4/7}$ IV & foliar spray	$Ca[NO_3]_2$ fertilizer 5/26	GA + NO_3 increased m & f, NO_3 alone increased f	Pollard and Portlock 1981b
T. heterophylla 7 & 8 yr. rooted ramets 3 & 6 yr. seedlings	$GA_{4/7}$ IV & foliar spray	$Ca[NO_3]_2$ fertilizer, H_2O stress on 3 yr. seedlings	ramets: $GA_{4/7}$ increased m (6-24X) $GA_{4/7}$ + NO_3 increased f (100X) 6 yr. seedlings f increased 47% 3 yr. seedlings m increased 7% f increased 30%	Ross et al. 1981
T. heterophylla 1-5 yr. seedlings	$GA_{4/7}$ foliar spray, weekly mid-June through July	H_2O stress	GA alone sparse flowering, GA + H_2O stress increased m & f 60% of 4 yr. seedlings flowered	Brix and Portlock 1982
T. heterophylla seedlings rooted cuttings	$GA_{4/7}$ foliar spray weekly	timing and duration: 1) 4/8-5/6 2) 4/8-6/3 3) 4/8-7/1 4) 4/8-7/9 5) 5/6-6/3 6) 6/3-7/1 7) 6/3-7/29 8) 7/1-7/29	cuttings - erratic, moderate number of m with 4 wk treatment seedlings - 12 wk treatments beginning April gave reliable production of m & f	Pollard and Portlock 1983
T. heterophylla	$GA_{4/7}$ foliar spray, weekly 5/15 - 6/15	H_2O stress	GA + H_2O stress increased m & f, synergistic for f, not m; significant clone x GA effect, 2nd yr. GA increased flowering & damage to tree	Rottink 1982

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
T. heterophylla rooted cuttings 3 yr. seedlings	GA$_{4/7}$ foliar spray 2-3 times/wk, 4/22 - 6/17	Fertilizer CA[NO$_3$]$_2$ 10g every 2 wks, photoperiod (PP), temperature (T) 3-yr. seedlings GA + Fert. +PP (13 or 18 hrs.) +T (20° or 30°C - constant) 4/22 - 6/17 rooted cuttings GA + Fert. +T (18°-32°C) 6/11-9/3 +Post-induction treatment 10/1-12/1: +PP(0 or 8 hrs.) decreasing+T (5.6 or 11.5°C)	3 yr. seedlings 18 hr. PP induced m 13 hr. PP induced f more m & f at 30° than 20°C rooted cuttings affected number of surviving strobili 0 hr PP + 11.1°C decreased the number of strobili surviving.	Pollard and Portlock 1984
		Cuppressaceae and Taxodiaceae		
Cryptomeria	GA$_3$ foliar spray 6/12, 7/14, 8/1		male female + +	Kato et al. 1959
Cupressus lawsoniana	same		+ +	
C. sempirvirens	same		+ +	
Thuja standishii	same		none +	
Thujopsis dolabrata	same		+ none	
Juniperus communis	same		+ none other species tested with no results	

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
Cryptomeria japonica	GA_3 foliar spray		male female + best +	Fukuhara 1961
Metasequoia glyptostrobides	same		+ +	
Taxodium distichum	same		+ +	
Other Taxodiaceae	same		little effect	
Cupressaceae	same		all except Chamaecyparis obtusa and C. pisifera showed effects - no difference in germination of pollen & seed from treated and non-treated C. japonica and C. lawsoniana	
Cryptomeria japonica 15 yr. old	GA_3, NAA, IAA, 2,4-D foliar spray	urea fertilizer pinching shoots	sex transition from m to f with GA + pinching, enhanced by other treatments	Hashizume 1961
Cryptomeria japonica Chamaecyparis obtusa	GA_3, ethrel foliar spray		GA increased f, enhanced by ethrel, inhibited GA effect on main stem elongation	Hashizume 1975
Cryptomeria japonica	GA_3 or $GA_{4/7}$ foliar spray, 3 times	12 combinations 3 day (20, 25 and 30°C) and 4 night (15, 20, 25 and 30°C) temp	most m with 30° day, 25° night most f with 25° day, 15° night	Nagao 1983b
Cupressus arizonica	GA_3 foliar spray starting at age 55 days		m initiated within 23-25 days of spraying, concentration required depended on age of seedling	Pharis et al. 1965; Owens and Pharis 1967

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
Cupressus pygmaea C. arizonica C. lusitanica C. funebris	GA$_3$ and/or GA$_{4/7}$ - foliar spray same same same		male female + + + + + + no response up to 100 days	Pharis and Morf 1967
C. arizonica	GA$_3$ foliar spray, 2 times/wk		2000mg/ℓ semi-toxic, number of strobili produced depended on time lapse between initial spray and last long day. Spraying 1 wk after last long day. Spraying 1 wk after last long day produced numerous m (10 times more lateral) meristems produced than under SD)	Pharis et al. 1970
C. arizonica Chamaecyparis lawsoniana	GA$_3$ ± ethrel, BA, NAA or CCC-foliar spray	Photoperiod (LD-SD-LD)	GA$_3$ increased m & f, ethrel enhanced	Bonnet-Masimbert 1971
C. nootkatensis	GA$_3$ foliar spray	natural LD (18 hr) SD (8 hr)	increased m & f, maximum m & f with LD + 4-5 wks GA	Owens and Moder 1977f
C. nootkatensis seedlings 3 yr. rooted cuttings	GA$_3$ weekly foliar spray, 2, 4 & 8 wk periods with midpoints of 7/9 or 8/6		treatments applied too late, only earliest applications started 6/11 resulted in f	Ross 1983b
Cupressus arizonica seedlings	GA$_{24}$ injected into small branches of 1-2 yr seedlings		increased flowering, low concentrations had no effect	Pharis et al. 1969
C. arizonica	GA$_3$ foliar spray 3 times/wk for 3 months, then 2 times/wk		vegetative damage occurred m & f formed in 7th month from seed	Fraser 1969

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
Sequoia sempervirens S. gigantea	GA_3, $GA_{4/7}$, GA_{13} topical to main stem	photoperiod 8, 12, or 16 hr.	Flowering at ≤ than 1 year needs 1500+ μg GA for S. gigantea, 1000+ μg GA for S. sempervirens 8 and 16 hr photoperiod effective	Pharis and Morf 1969
Thuja occidentalis	GA_3 foliar spray 3 times/wk for 3 months, then 2 times/wk		decreased apical growth in young seedlings, increased apical growth in seedlings 25 cm tall, m & f formed in 7th month from seed	Fraser 1969
T. occidentalis	GA_3 foliar spray	long photoperiod	increased f	Fraser 1970
T. plicata	GA_3 foliar spraying	±photoperiod (LD-SD-LD)	male + female + (m flowered without LD-SD-LD but aborted before mature)	Pharis and Morf 1967
T. plicata	GA_3 biweekly foliar spray	photoperiod	(LD-SD-LD) + GA_3 increased f	Pharis et al. 1969
T. plicata	GA_3 foliar spray	photoperiod 16 hr 8 hr	GA_3 100ppm + 16 hr increased m GA_3 500ppm + 8 hr increased f	Pharis and Morf 1970
T. plicata	GA_3 foliar spray 2 times/wk for 34 wks.	16 wks LD warm, 7 wks SD cold, 11 wks LD warm,	increased m & f	Owens and Pharis 1971
T. plicata	GA_3 IV	ringing	no increase in m, GA, GA + ringing, ringing all increased f	Coutts and Bowen 1973

Appendix 8 (cont.)

SPECIES	GR TREATMENT	OTHER TREATMENT	EFFECT	REFERENCE
T. plicata	GA$_3$, NAA, IAA, KI, ABA, BAP, ethrel added to nutrient medium	Photoperiod (SD), *in vitro* culture of vegetative shoot tips	increased m but prolonged culture resulted in externally mature strobili but sporogenous tissue either aborted or arrested at meiosis. No f	Coleman and Thorpe 1978
T. plicata seedlings 3 yr. rooted cuttings	GA$_3$ foliar spray 2, 4 & 8 week periods with midpoints of 7/9, 8/6 biweekly & weekly branch applications with aerosol atomiser		f formed with all GA treatments, early July to early Aug. was best for increased f initiation, but should extend treatment to mid-Aug. for proper cone development. 4 wks from early June to early July was best form	Ross 1983b